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COMPARATIVE STUDIES OF CERTAIN CULTURES OF PUCCINIA RUBIGO-VERA AND PUCCINIA TOMIPARA ON WILD GRASSES[1]

GEORGE W. FISCHER[2]

(Accepted for publication November 26, 1934)

INTRODUCTION

The leaf rust of grasses, *Puccinia rubigo-vera* (DC.) Wint. probably is the most composite rust species known from the standpoint of the phenomena of host specialization. Mains (24) distinguished 56 races within this species, one specialized to rye, one to wheat, and 54 to combinations of wild-grass hosts. Physiologic specialization has been most extensively studied in the races on the cereals. Within the rye race, *Puccinia rubigo-vera* sp.f. *secalis* (Eriks. and Henn.) Carleton (*Puccinia dispersa* Eriks.) two physiologic forms have thus far been differentiated by Mains (22). Within the wheat race, *Puccinia rubigo-vera* sp.f. *tritici* (Eriks.) Carleton (*Puccinia triticina* Eriks.) at least 53 physiologic forms are known to exist. These are distinguished by their reaction to a differential set of agronomic varieties of wheat, (25), (15).

In the wild grasses there has not been such a selection and differentiation of varieties as has occurred in cultivated grasses. However, this does not exclude the possibility of the existence within wild-grass species of strains that differ in their reaction to rust.

It was the purpose of the investigations here presented to make a study of several cultures of *Puccinia rubigo-vera*, and of the wild grasses that serve as their hosts, in an effort to obtain some information concerning the extent to which the physiologic specialization of these cultures is comparable to the specialization of the leaf rust of cereals.

[1] Papers from the Department of Botany and the Herbarium of the University of Michigan no. 504.

[2] The writer very gratefully acknowledges the helpful criticism and advice accorded him by Dr. E. B. Mains, under whose supervision these investigations were conducted. He is also grateful to the personnel of the Botanical Gardens of the University of Michigan for the facilities placed at his disposal and for the many courtesies shown.

HISTORICAL SKETCH

The history of *Puccina rubigo-vera* began in 1815, when de Candolle (6) gave the name *Uredo rubigo-vera* to a rust on wheat, in France. Winter (36) in his treatment of the rusts in Rabenhorst's "Kryptogamen-Flora," assigned this species to the genus Puccinia and recognized that it occurred on a number of grasses. With the development of knowledge concerning host restriction, especially with regard to aecial host, various groups have been segregated as species, races, forms, etc.

Thirty-five botanists have proposed 65 names for segregates of *Puccinia rubigo-vera.* Arthur and Fromme (4) have distributed these under 5 rusts: *Dicaeoma Asperifolii* (Pers.) Kuntze, *D. apocryptum* (Ellis and Tracy) Kuntze, *D. procerum* (Dietel and Holway) Kuntze, *D. Cockerellianum* (Bethel) Arthur and Fromme, and *D. Clematidis* (DC.) Arthur. These species differ chiefly in having aecial hosts belonging to different families of plants. Recently, however, Mains (24) united these species and recognized a number of more or less sharply differentiated races, in the resulting composite species, *Puccinia rubigo-vera.* These races were named by Mains (*op. cit.*) and separated by virtue of aecial and telial host combinations and grass host ranges. According to this concept of *Puccinia rubigo-vera,* 86 species of plants, representing 25 genera, and 4 families, serve as aecial hosts for the 56 known physiologic races of this rust species. More than 90 species in 16 genera of grasses serve as uredial and telial hosts. These are distributed in 4 tribes, *viz.*, Agrostideae, 2 races; Avenae, 3 races; Festuceae, 14 races; and Hordeae, 38 races. Still more recently, Arthur (5), in his "Manual of the Rusts in United States and Canada," has recognized this composite species, *Puccinia rubigo-vera,* but divides it into 6 varieties on the basis of aecial and telial host combinations, aided in some cases by spore size and geographic distribution. A complete history of *Puccinia rubigo-vera,* and an organization of the synonymy have been given by Mains (24).

The race restricted to wheat, *Puccinia rubigo-vera,* sp.f. *tritici* (Eriks.) Carleton, has been studied most extensively, especially with regard to physiologic specialization. Mains and Jackson (25) were able to distinguish 12 physiologic forms of the leaf rust of wheat in the United States by the differential reaction of 11 agronomic varieties. Since then, contributions by Scheibe (27) (28), Waterhouse (33), Johnston (14), Johnston and Mains (15), and others have brought the number of known forms up to 53. That some of these forms can be further divided into sub-forms by the use of additional differential wheat varieties has been shown by Waterhouse (33) (34), and Scheibe (28).

No wild-grass race of *Puccinia rubigo-vera* has been studied so inten-

sively; as a result, comparatively little is known regarding host specialization within the wild-grass races.

A few earlier investigators, working with cultures of wild-grass races of *Puccinia rubigo-vera* have noticed differences in reaction between different collections of wild-grass species.

Thus Mains (24, P. 384) found that,

> one collection of *Hordeum jubatum* apparently was resistant to culture Th. 15 of the race *vulgaris* (Table II) and to race *occidentalis* (Table III) and susceptible to other cultures of these races. Likewise one collection of *Elymus virginicus* was apparently resistant to one culture of race *anomala* (Table II) and to one of race *Impatientis* (Table XIII), though susceptible to the other cultures of these races. Though these instances are hardly sufficient to justify conclusions, they suggest that some at least of the wild-grass races may be subdivided through differences in reaction of selections or strains of the various species of grasses.

Mains (*op. cit.* P. 384) further asserts,

> There is no reason to assume that the races with wild-grass hosts differ in this respect from the race *Tritici* on wheat. The last has received extensive study in this regard, but too few collections of both the wild-grass races and their hosts have been studied to justify a comparison.

Mains (23) noted strain differences in resistance and susceptibility in the hosts of *Puccinia dispersa* Eriks. (*Puccinia rubigo-vera* sp.f. *secalis* (Eriks. and Henn.) Carleton), *P. agropyrina* Eriks. (*P. rubigo-vera* sp.f. *persistens* (Plowright) Mains), *P. montanensis* Ellis, and *P. andropogonis* Schw.

Hassebrauk (12), obtained some very interesting results in his studies of the reactions of collections of numerous wild grasses to the several cereal rusts. Besides finding that in many cases the wild grasses are susceptible to the particular forms of cereal rusts that he used, he observed many cases in which a number of collections of the same grass species from different sources behaved quite differently as to rust reaction. For instance, one of his two collections of *Elymus virginicus* was fairly susceptible to form 14 of *Puccinia triticina* Eriks. (*P. rubigo-vera* sp.f. *tritici*), whereas the other was resistant. The two collections had come from widely separated sources.

In this investigation it was thought best to employ a few races of *Puccinia rubigo-vera* and a number of species of grasses. Several collections of each host species, usually from different localities, were obtained in order to study the possibility of reactional differences within grass species.

The investigations of *Puccinia tomipara* are a natural outgrowth of the studies of *P. rubigo-vera* since the former has been considered a race of the latter (3). It differs from *P. rubigo-vera* in producing multicellular telio-

spores. *P. tomipara* was described by Trelease (32) who found it infecting *Bromus ciliatus* L. Arthur (1) considered this to be merely a morphologic variation sometimes met in grass rusts, and when he (1) connected aecia on *Clematis virginiana* L. with telia of the leaf rust type on *Bromus ciliatus*, having normal two-cell teliospores, he therefore used the name *P. tomipara* for this rust. He also used this name again (2) in designating a successful infection of *C. virginiana* from two-cell teliospores from *B. ciliatus*. It is to be noted that these Clematis-Bromus connections were really obtained with typical collections of *P. rubigo-vera*.

Fraser (10) was the first to establish the true aecial connection of *Puccinia tomipara*. Field evidence suggested the connection of aecia on *Thalictrum dasycarpum* Fisch. and All. with leaf rust on *Bromus ciliatus* and species of Elymus and Agropyron. When he inoculated these grasses with aeciospores from *T. dasycarpum*, heavy infection resulted on *Elymus canadensis* L., *E. virginicus* L. and *B. ciliatus* with uredia and telia in great abundance. Microscopic examination of the telia ultimately resulting from these inoculations showed those on the *Elymus* spp. to be of the normal two-cell leaf rust type. The teliospores taken from *B. ciliatus*, however, were

> very variable in size, shape and number of cells, only rarely could two-celled spores of the Puccinia type be found, practically all of the spores being three to several celled, some having as many as sixteen cells.

Fraser (10) inoculated *Elymus virginicus*, *Agropyron tenerum* Vasey, *A. Smithii* Rydb., *A. repens* (L.) Beauv. and *Hordeum jubatum* L. with this Bromus rust but no infection resulted. This rust was clearly *Puccinia tomipara*. Fraser stated that the multicellular character seemed fixed, inasmuch as many collections, both on *Bromus ciliatus* and *B. latiglumis* (Shear) Hitch. (*B. altissimus* Pursh.), showed this character. He considered it best to include the rust under *P. agropyri* E. and E. until further study established its true position. Fraser also stated that it seemed doubtful whether the rust should even be included in the genus Puccinia.

Arthur *et al* (3) considered *Puccinia tomipara* to be a teratologic race, into which category they also put the related species, *P. elymi* Westend., a multicellular form on Elymus, in which the cells of the teliospores are arranged in linear fashion.

Lagerheim (18) erected the genus Rostrupia on the strength of Westendorp's (35) *Puccinia elymi* and Trelease's *P. tomipara*, considering the multicellular character of the teliospores of these rusts to be a good generic character. Since then several rusts have been assigned to this genus by Dietel (7), but Arthur *et al.* (4), in the North American Flora, reduced these species to synonymy insofar as they apply to North American rusts. Accordingly, *P. elymi* and *P. tomipara* are listed by Arthur *et al.* (4) in the

synonymy for *Dicaeoma clematidis* (DC.) Arth. Mains (24), on the other hand, did not include these two grass rusts in *P. rubigo-vera*, and stated that it seemed best, for the time being, to regard them as distinct species until culture work has shown whether the multicellular character is or is not stable. More recently, Arthur (5) in his "Manual of the Rusts in United States and Canada," cites *P. tomipara* and *P. elymi* as synonyms of *P. rubigo-vera*.

MATERIALS AND METHODS

In these studies 111 collections of species and varieties of wild grasses were used; 65 belong to the genus Bromus; 46 belong to the tribe Hordeae, including 20 numbers of 9 species and varieties of Elymus, 7 numbers of 4 species of Agropyron, 17 numbers of 5 species of Hordeum and 1 number of *Hystrix patula* Moench. It will be noted that in many instances the various species are represented by more than a single collection.[3]

Although 20 collections of *Puccinia rubigo-vera* were studied to some extent, only 9 of these received concentrated study and are reported here. One culture of *P. tomipara* also is included. These rust cultures are referred to in the following pages by numbers only.

The aecial hosts of most of the cultures were determined as a result of clues obtained from field associations. Thus cultures 1, 13, and 20 were connected with Thalictrum; 14, 18, 21 with Impatiens; 22 and 23 with Clematis; connections of cultures 8 and 11 were not determined. Herbarium specimens of the various cultures have been deposited in the herbarium of the University of Michigan.

In Table 1 is given a summary of all the field data concerning these cultures of *Puccinia rubigo-vera* and of *P. tomipara*.

The inoculation methods used in the present investigations have been described by Mains (21). Minor modifications are explained below.

On the most susceptible grass for each rust culture, attempts were made to build up a supply of inoculum with which to inoculate a series of grasses. If these "stock cultures" were watered from below for a few days prior to use for inoculation, the urediospores piled up on each uredium, resulting in the accumulation of a considerable supply of inoculum.

In the process of inoculation, in addition to shaking the heavily rusted "stock plants" over the previously atomized grasses, it was found advan-

[3] The numbers appended to the grass collections are the accession numbers assigned to them by Dr. E. B. Mains who was instrumental in obtaining most of them, especially from foreign botanical gardens. Numbers preceded by B. G. are accession numbers of the Botanical Garden of the University of Michigan. Acknowledgement and thanks are due Dr. J. R. Swallen, of the Smithsonian Institution, and to Prof. J. H. Ehlers of the Department of Botany of the University of Michigan, for checking the identification of the grasses used.

TABLE 1.—*Cultures of Puccinia rubigo-vera obtained from Michigan, Indiana, Ohio and Kansas and of P. tomipara from Michigan*

Culture	Hosts in Field		Where Collected	Collector
	Aecial	Telial		
		Puccinia rubigo-vera		
1	*Thalictrum*[1] *spp.*	*Agropyron repens*	Ann Arbor, Mich.	G. W. Fischer
8	undetermined	*Agropyron repens*	Angola, Ind.	G. W. Fischer
11	undetermined	*Elymus virginicus*	Manhattan, Kansas	C. O. Johnston
13	*Thalictrum dioicum*	*Agropyron tenerum*	Pinckney, Mich.	G. W. Fischer
14	*Impatiens biflora*	*Elymus virginicus*	Ann Arbor, Mich.	G. W. Fischer
18	*Impatiens biflora*	*Elymus virginicus*	Adam's Mills Ohio	E. B. Mains
21	*Impatiens biflora*	*Elymus virginicus* *Elymus striatus*	Ann Arbor, Mich.	G. W. Fischer
22	*Clematis virginiana*	*Elymus virginicus*	Marquette, Mich.	E. B. Mains
23	*Clematis virginiana*	*Bromus ciliatus* *Bromus altissimus*	Pinckney, Mich.	G. W. Fischer
		Puccinia tomipara		
20	*Thalictrum dasycarpum, T. dioicum*	*Bromus ciliatus* *B. altissimus*	Ann Arbor, Mich.	G. W. Fischer

[1] Determined by inoculation experiments only

tageous to draw these back and forth over the wet grasses very much after the manner in which one would use a paint brush. A more satisfactory distribution of inoculum was effected in this manner than by any other method tried. After inoculation the whole was then incubated 12 to 24 hours under 2 or 3 layers of wet muslin. When uncovered, the grasses were always wet with condensed moisture, thus simulating dew in the field.

Uredia usually began to appear in 8 to 10 days on the most susceptible grasses, but data were not taken until at least 2 weeks after inoculation in order to allow ample time for full expression of reaction. A card file of the grasses proved to be convenient when taking data.

In order to avoid contaminations of one rust culture with another, each was kept, together with its individual series of grasses, in a separate compartment of a portion of the greenhouses especially constructed for rust and mildew investigations at the Botanical Gardens of the University of Michigan.

For inoculation purposes seedlings of the grasses were used, for, in

this stage of growth, the hosts would naturally be in a vigorous condition and a more reliable rust reaction would be secured. Grasses to be inoculated were grown in 2½-inch pots, with 5 to 15 seedlings in each, and were ready for inoculation after 6 or 7 weeks. It is recognized that, in some cases at least, susceptibility reported here for grasses in the seedling stage does not necessarily imply a similar susceptibility in the field or when mature. It has been noted by previous investigators of grass and cereal rusts, notably Johnston and Melchers (16), and Stakman and Piemeisel (30), that certain varieties or strains of the host plant might show a high degree of susceptibility in the seedling stage, but be highly resistant at heading time.

Although it was often found that the types of reaction of the wild grasses to leaf rust are not so clearly defined as are those to the leaf rusts of cereals (a fact also reported by Mains (24)), the symbols used in designating such reactions on cereals have, nevertheless, been found convenient. They are those employed by Stakman and Levine (29) for stem rust, *Puccinia graminis*, and by Mains and Jackson (25) in their studies on the leaf rust of wheat, modified slightly for adaptation to the present studies as follows:

0. Highly resistant	No uredia produced; various degrees of flecking produced in some cases, entirely absent in others.
1. Very resistant	Uredia few, very small, often abortive; in some cases accompanied by pronounced necrosis; necrotic spots often without uredia.
2. Moderately resistant	Uredia better developed than in the preceding; necrotic spots present but less pronounced and not without uredia.
3. Moderately susceptible	Uredia well developed, of moderate size; never accompanied by necrotic areas.
4. Very susceptible	Uredia relatively large, well-developed, and capable of copious production of urediospores.

The method used in inoculating aecial hosts is the standard one whereby the overwintered telia, under the proper conditions of temperature and moisture, are suspended over the plants to be inoculated. For the most part the plant cover recommended by Mains (21), consisting of a wooden "house" with a glass "roof," was used. The telia of the rust cultures were overwintered in loose cheese-cloth bags or loosely wrapped in mosquito netting. In the spring, as soon as the teliospores showed sufficient germination, inoculations of the aecial host were begun.

RESULTS

Cultural Experiments with *Puccinia rubigo-vera*

Culture 1. On Agropyron repens and Thalictrum spp.—This culture was collected on couch or quack grass, *Agropyron repens*, at the Botanical

Garden of the University of Michigan, and represents a leaf rust that is very prevalent throughout the lower peninsula of Michigan.

Culture 1 was especially adapted to greenhouse study. It was easily propagated in the uredial stage on either seedlings of quack grass or on plants grown from potted rhizomes from the field. Unlike most of the other rust cultures studied, culture 1 produced telia in very scant quantities.

This culture of leaf rust of *Agropyron repens* is very like that found in England on the same host and described by Plowright (26) as *Puccinia persistens,* or the rust designated by Eriksson and Henning (9) as *P. dispersa* f. *agropyri,* and that Eriksson (8) later raised to specific rank as *P. agropyrina.* Mains (24) considered that these names all refer to one race of *P. rubigo-vera.* This he designated as *P. rubigo-vera* sp.f. *persistens* (Plowright) Mains.

Inoculations of a series of grasses during the past two years have given the following results:

DISTINCTLY SUSCEPTIBLE (reaction type 3–4): *Agropyron dasystachyum* (Hook.) Scrib. (1656), *A. repens* (L.) Beauv. (1467, B.G. 13007), *A. tenerum* Vasey (1484, 1659), *Elymus glaucus* Buckley (1539), *Hordeum gussoneanum* Parl. (817, 1393, 1421, 1457), *H. jubatum* L. (1647), *H. murinum* L. (1548).

MODERATELY SUSCEPTIBLE (reaction type 2–3): *Elymus canadensis* L. (1640), *E. virginicus* L. (1793), *Hordeum gussoneanum* (1291, 1433), *H. murinum* (1510), *H. pusillum* Nutt. (1650).

DISTINCTLY RESISTANT (reaction type 0–2): *Agropyron caninum* (L.) Beauv. (1466, B.G. 12770, 803), *Elymus glaucus* (1482), *E. robustus* (S. and S.) Mack and Bush (1642), *E. striatus* Willd. (1645), *E. striatus arkansanus* (S. and B.) (Hitchc. (780)), *E. virginicus* (765, 842, 1475, 1476, 1538), *E. virginicus glabriflorus* (Vasey) Bach. (1646), *E. virginicus submuticus* Hook. (793), *Hordeum murinum* (743, 785, 1290, 1434, 1458), *H. nodosum* L. (1509), *Hystrix patula* (1477).

Plowright (26) showed that the leaf rust of *Agropyron repens* in England produces its aecia on *Thalictrum flavum* L. and possibly *T. minus* L. Treboux (31) reported having connected aecia on *Clematis Pseudoflammula* with uredia and telia on *A. repens.* Mains (24) reported aecia having been produced on *T. delavayi* Franchet, *T. flavum* and *T. minus* from telia of the leaf rust of *A. repens.*

In the present experiments, overwintered telia of *Puccinia rubigo-vera* on *Agropyron repens* suspended over species of Thalictrum in an inoculating chamber, resulted in the production of pycnia and aecia on *Thalictrum minus, T. glaucum, T. flavum* and *T. fendleri.* No infection resulted on *T. dioicum* L., and *T. polygamum.* Similar inoculations of *Clematis virginiana* and *Impatiens biflora* Walt. yielded negative results.

Jackson and Mains (13) pointed out the evident relationship of the leaf rust of *Agropyron repens* to the leaf rust of wheat, emphasizing the fact that they have similar aecial hosts and are morphologically similar. The cultural behavior of the former in the greenhouse is strikingly like that of the latter.

Culture 8. On Agropyron repens (Aecial Host Undetermined). This culture obviously is related to Culture 1, but is physiologically distinct. It was collected at Angola, Indiana, on *Agropyron repens.* No telia were present, and since there was only a very scant production of these sori in the greenhouse, no attempt was made to determine aecial-host connections.

Inoculations of a series of grasses with culture 8 have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron repens* (1467), *Elymus canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1482, 1539), *Hordeum gussoneanum* (817, 1393, 1421, 1433, 1457), *H. murinum* (1548).

MODERATELY SUSCEPTIBLE: *Agropyron tenerum* (1489), *Elymus virginicus* (1475), *Hordeum murinum* (1510).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770, 1466), *A. repens* (B.G. 13007), *Elymus canadensis* (843), *E. striatus arkansanus* (780), *E. virginicus* (842, 1476, 1538), *E. virginicus submuticus* (793), *Hordeum gussoneanum* (1292), *H. murinum* (743, 785, 1290, 1434, 1458), *H. nodosum* (1435), *Hystrix patula* (1477).

It will be seen from the above data that cultures 8 and 1 differ chiefly in the marked susceptibility to the former of certain collections of *Elymus canadensis.*

Culture 11. On Elymus virginicus (Aecial Connection Undetermined). This culture came from Manhattan, Kansas, where C. O. Johnston collected it on *Elymus virginicus.* It was easily and readily multiplied on plants of *E. virginicus* in any stage of development, so that an abundant supply of inoculum was produced. Telia were rarely produced and then in such scant quantities that no attempt was made to determine the aecial host connections.

Inoculations of a series of grasses with culture 11 over a period of 2 years have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron dasystachyum* (1656), *A. tenerum* (1484, 1659), *Elymus canadensis* (795, 1305, 1366, 1640), *E. glaucus* (1539), *E. robustus* (1642), *E. virginicus* (765, 842, 1475, 1476, 1793), *E. virginicus glabriflorus* (1646), *E. virginicus submuticus* (793), *Hordeum murinum* (1548), *Hystrix patula* (1477).

MODERATELY SUSCEPTIBLE: *Elymus glaucus* (1482), *E. striatus arkansanus* (780), *E. virginicus* (1538).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. repens* (1467, B.G. 13007), *Elymus australis* (1644), *E. canadensis* (843, 1537), *E. striatus* (1645), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1510), *H. nodosum* (1435, 1509).

From these results it is seen that this culture of *Puccinia rubigo-vera* finds favorable hosts in a rather wide range of grasses. Eighteen collections of 8 species and 2 varieties, representing 4 genera, have proved to be distinctly susceptible.

Culture 13. On Thalictrum dioicum and Agropyron tenerum. On May 28, 1932, the writer found, near Pinckney, Michigan, plants of *Thalictrum dioicum* heavily infected with aeria. A considerable quantity of this material was collected and used to inoculate a series of grasses.

An abundance of plants of *Agropyron tenerum* and *Elymus virginicus*, heavily infected with telia of leaf rust, were collected in the same locality the following September. These were put outside to hibernate, but each host was kept separately.

In the spring of 1933, the overwintered telia on *Agropyron tenerum* were used to inoculate plants of *Impatiens biflora, Clematis virginiana, Thalictrum dioicum, T. dasycarpum, T. flavum*, and *T. glaucum*. Aecia were produced on the *Thalictrum* spp. only. Another set of the same plants were inoculated from the telia overwintered on *Elymus virginicus;* aecia resulted on *Impatiens biflora* only. This Impatiens-Elymus culture was not further studied.

Inoculations with this culture are not complete. However, the series of grasses inoculated gave the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron tenerum* (1484, 1659), *Elymus canadensis* (795, 1305, 1366), *E. glaucus* (1482), *E. virginicus* (842, 1475), *Hordeum gussoneanum* (1433), *H. murinum* (1548).

MODERATELY SUSCEPTIBLE: *Elymus virginicus* (1476), *Hordeum gussoneanum* (1457), *Hystrix patula* (1477).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. repens* (1467, B.G. 13007), *Hordeum gussoneanum* (817, 1421), *H. murinum* (743, 785, 1434), *H. nodosum* (1435).

Apparently this is the first demonstration of the connection of *Puccinia rubigo-vera* on *Agropyron tenerum* with *Thalictrum* spp. either by culture experiments or field observations.

Culture 14. On Elymus virginicus and Impatiens biflora. This culture of leaf rust originated from a collection made by the writer on *Elymus virginicus*, northwest of Ann Arbor, in September, 1931. Uredia and telia were present, the latter in abundance; both were collected. The uredio-

spores were sown on seedlings of *Elymus virginicus* in the greenhouse, giving rise to uredia in 9 to 10 days. The telia were not successfully overwintered. In the fall of 1932 more telia were collected from the same region. These were overwintered successfully, and good germination was obtained the following spring. This collection was used to inoculate *Clematis virginiana, Thalictrum dioicum,* and *Impatiens biflora;* pycnia and aecia were produced on the last, only.

The greenhouse culture of the rust on *Elymus virginicus* seedlings was multiplied with fair success for a few weeks. However, during the months of November, December, and January, the uredial culture was nearly lost, the uredia being gradually supplanted by telia. It was not until after February that this culture could be multiplied in the uredial stage.

Repeated inoculations with culture 14 of *Puccinia rubigo-vera* yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron caninum* (1466), *A. dasystachyum E. robustus* (1642), *E. striatus arkansanus* (780), *E. virginicus* (765, 842, (1656), *A. tenerum* (1484), *Elymus canadensis* (795, 1305, 1366, 1640), 1475, 1793), *E. virginicus submuticus* (793), *Hystrix patula* (1477).

MODERATELY SUSCEPTIBLE: *Elymus glaucus* (1539), and *E. virginicus* (1476).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770), *A. tenerum* (1659), *A. repens* (1467, B.G. 13007), *Elymus australis* (1644), *E. canadensis* (843, 1537), *E. glaucus* (1482), *E. striatus* (1645), *E. virginicus* (1538), *E. virginicus glabriflorus* (1646), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1548), *H. nodosum* (1435, 1509), *H. pusillum* (1650).

Culture 18. On Elymus virginicus and Impatiens biflora. This culture of leaf rust came from a collection of telia on *Elymus virginicus* made by E. B. Mains, at Adams Mills, Ohio, September, 1931. These telia were overwintered in the usual manner and a good germination of the teliospores resulted. These were used to inoculate potted plants of *Impatiens biflora, Thalictrum fendleri, T. dasycarpum, T. flavum, T. glaucum, Ranunculus acer, Anemone virginiana* and *Clematis virginiana.* Pycnia and aecia developed on *I. biflora,* only, from which infection the aeciospores were sown on seedlings of *E. virginicus* (842, 1475). A vigorous development of uredia resulted. The culture was multiplied and the resulting inoculum oversummered at a temperature of 10° to 12° C. The following October the culture was renewed from these stored urediospores. A fair infection on *Elymus virginicus* resulted. The uredia gradually reverted to telia until, by the middle of December, the uredial culture was lost entirely. Abundant telia, however, were produced and overwintered and the culture was renewed the

following spring (1932) from inoculations on *Impatiens biflora*. Grass inoculations were carried out in early summer.

Inoculations of a series of grasses with culture 18 have yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron tenerum* (1659), *A. dasystachyum* (1656), *Elymus australis* (1644), *E. canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1539), *E. striatus* (1645), *E. virginicus* (765, 842, 1475, 1476, 1538, 1793), *Hordeum nodosum* (1509).

MODERATELY SUSCEPTIBLE: *Agropyron caninum* (1466), *Elymus robustus* (1642), *E. virginicus glabriflorus* (1646), *Hordeum jubatum* (1647).

DISTINCTLY RESISTANT: *Agropyron caninum* (B.G. 12770), *A. repens* (1467, B.G. 13007), *Elymus canadensis* (1640), *Hordeum gussoneanum* (817, 1393, 1421, 1433, 1457), *H. murinum* (743, 785, 1290, 1434, 1458, 1510, 1548), *H. pusillum* (1650).

Culture 21. On Elymus striatus and Impatiens biflora. Culture 21 of leaf rust came from a collection of uredia and telia on *Elymus striatus* that the writer made 5 miles south of Ann Arbor in October, 1932. The uredia were used to start the culture on plants of *E. striatus* brought back from the locality where the rust was collected. The telia were put outside to overwinter. They germinated well the following spring and were used to inoculate plants of *Impatiens biflora*, *Clematis virginiana*, *Thalictrum dioicum*, *T. dasycarpum*, *T. glaucum*, *T. flavum*, and *T. minus*. Pycnia and aecia were produced on *I. biflora*, only. The aeciospores on Impatiens were used to start the culture from which the grass inoculations were made.

Inoculations of a series of grasses with culture 21 yielded the following results:

DISTINCTLY SUSCEPTIBLE: *Elymus canadensis* (795, 843), *E. robustus* (1642), *E. virginicus* (842, 1476, 1793), *E. virginicus* var. *submuticus* (793).

MODERATELY SUSCEPTIBLE: *Elymus canadensis* (1537, 1640), *E. glaucus* (1539), *E. striatus* var. *arkansanus* (780), *E. virginicus* (1475), *Hordeum gussoneanum* (1421), *H. pusillum* (1650).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, 1466, B.G. 12770), *A. dasystachyum* (1656), *A. repens* (1467, B.G. 13007), *A. tenerum* (1659), *Elymus australis* (1644, *E. canadensis* (1305, 1366), *E. glaucus* (1482), *E. striatus* (1645), *E. virginicus* (765, 1538), *E. virginicus* var. *glabriflorus* (1646), *Hordeum gussoneanum* (817, 1291, 1393, 1433, 1457), *H. jubatum* (1647), *H. murinum* (743, 785, 1290, 1434, 1458, 1510, 1548), *H. nodosum* (1509), *Hystrix patula* (1477).

It is interesting to note that the *Elymus striatus* on which culture 21 was collected and later propagated was highly susceptible, while the *E.*

striatus (1645) from Indianapolis, Indiana, was distinctly resistant. Only moderate susceptibility was shown by the variety *arkansanus* (780) of *E. striatus*. Furthermore, culture 21 is the only culture of *Puccinia rubigo-vera* studied (culture 23 on *Bromus* spp., excepted) that had no susceptible hosts in the genera Agropyron and Hordeum.

Culture 22. On Clematis virginiana and Elymus virginicus. Culture 22 of *Puccinia rubigo-vera* was obtained from aecia collected on *Clematis virginiana* by E. B. Mains, at Marquette, Michigan, June, 1933. The aeciospores, sown on *Elymus virginicus* (1475) and *Bromus ciliatus* (1649), resulted in a heavy infection on *E. virginicus*, only. The culture was multiplied twice, and abundant inoculum was obtained to inoculate the usual series of grasses. Inoculation experiments were carried out during the summer.

Inoculations of the following grasses with culture 22 have given the following results:

DISTINCTLY SUSCEPTIBLE: *Agropyron caninum* (1466), *A. tenerum* (1659), *Elymus canadensis* (1305, 1537), *E. glaucus* (1539), *E. robustus* (1642), *E. virginicus* (765, 842, 1475, 1793), *Hordeum jubatum* (1647), *H. nodosum* (1509), *H. pusillum* (1650).

MODERATELY SUSCEPTIBLE: *Hordeum murinum* (1434, 1458, 1510).

DISTINCTLY RESISTANT: *Agropyron caninum* (803, B.G. 12770), *A. repens* (1467, B.G. 13007), *A. dasystachyum* (1656), *Elymus australis* (1644), *E. canadensis* (795, 843, 1366, 1640), *E. glaucus* (1482), *E. striatus* (1645), *E. striatus arkansanus* (780), *E. virginicus* (1476, 1538), *E. virginicus glabriflorus* (1646), *E. virginicus submuticus* (793), *Hordeum gussoneanum* (817, 1291, 1393, 1421, 1433, 1457), *H. murinum* (743, 785, 1290, 1548), *H. nodosum* (1435), *Hystrix pastula* (1477).

Table 2 summarizes the reactions of the 46 collections of species of Agropyron, Elymus, Hordeum, and Hystrix to the 8 cultures of *Puccinia rubigo-vera* described in the foregoing.

Culture 23. On Bromus ciliatus and Clematis virginiana. This culture was obtained accidentally while working with *Puccinia tomipara*. Overwintered telia on *Bromus ciliatus* collected near Pinckney, Michigan, in September, 1932, were being used to inoculate plants of *Thalictrum dioicum* and *T. dasycarpum* (April 29, 1933). A healthy potted plant of *Clematis virginiana* was also placed in the inoculation chamber along with the *Thalictrum* spp.

Pycnia appeared, not only on the *Thalictrum* spp., as expected, but also on *Clematis virginiana*, followed by aecia on both. Thus it appeared as if *Puccinia tomipara* produced aecia on *C. virginiana*, as well as on species of Thalictrum. The aeciospores from *C. virginiana* were used to inoculate

TABLE 2. *Summary of the reactions of 46 collections of wild-grass species to 8 cultures of Puccinia rubigo-vera*

Wild-grass collections	Cultures of *Puccinia rubigo-vera*							
	1	8	11	13	14	18	21	22
	TYPES OF REACTION							
Agropyron caninum 803	0	0	0	0	0	0	0	0
A. caninum 1466	0	0	0	.....	3–4	3	2	3–4
A. caninum B.G. 12770	0	0	0	0	0	0	0	1–2
A. dasystachyum 1656	4	.....	4	.....	3–4	3–4	0	0
A. repens 1467	3–4	3–4	0	0	0	0	0	0
A. repens B.G. 13007	3–4	0	0	0	0	0	0	0
A. tenerum 1484	3–4	3	4	3–4	4	.....	.....	.....
A. tenerum 1659	3–4	.....	3–4	4	0	3–4	0	3–4
Elymus australis 1644	0	.....	0	.....	0	3–4	0	0
E. canadensis 795	0	3–4	4	3–4	3–4	.....	4	0
E. canadensis 843	0	0	0	0–1	0	.....	3–4	0
E. canadensis 1305	0	3–4	4	3–4	3–4	4	0	4
E. canadensis 1366	0	3–4	3–4	3–4	3–4	3–4	0	0
E. canadensis 1537	0	3–4	0	0	0	3–4	3	3–4
E. canadensis 1640	2–3	.....	3–4	.....	3–4	2	3	0
E. glaucus 1482	0	4	3	4	0	.....	0	0
E. glaucus 1539	3–4	4	4	.....	3	3–4	3	3–4
E. robustus 1642	0	.....	3–4	.....	4	2–3	3–4	3–4
E. striatus 1645	0–2	.....	0	.....	0	3–4	0	0
E. striatus var. *arkansanus* 780	0	0–1	3	.....	3–4	3–4	3	0
E. virginicus 765	0	.....	3–4	2	3–4	3–4	0	4
E. virginicus 842	0	0	4	3–4	4	4	3–4	3–4
E. virginicus 1475	0	3	4	3–4	3–4	3–4	3	4
E. virginicus 1476	0	0	3–4	3	3	3–4	3–4	0–1
E. virginicus 1538	0	0	2–3	0	0	3–4	0	0
E. virginicus 1793	2–3	.....	4	.....	3–4	3–4	3–4	4
E. virginicus var. *glabriflorus* 1646	0	.....	3–4	.....	0	3	0	0
E. virginicus var. *submuticus* 793	0	0	3–4	.....	3–4	.....	3–4	0
Hordeum gussoneanum 817	3–4	4	0	0	0	0	0	0
H. gussoneanum 1291	2–3	0	0	.....	0	.....	0	0
H. gussoneanum 1393	3–4	3–4	0	.....	0	0	1–2	1–2
H. gussoneanum 1421	3–4	3–4	0	0	0	0	3	1–2
H. gussoneanum 1433	2–3	3–4	0	3–4	0–1	0	0	0
H. gussoneanum 1457	3–4	4	0	3	0	0	0	2
H. jubatum 1647	4	.....	0	.....	0	2–3	0	3–4
H. murinum 743	0–2	0–1	0	0	0	0	0	0–2
H. murinum 785	0–2	0	0–2	0	0	0	0	2
H. murinum 1290	0–2	0	0–2	.....	0	0	0	0–2
H. murinum 1434	0–2	0–2	0–2	0	0	0–1	0	2–3
H. murinum 1458	0–2	0	0–2	.....	0	.....	0	2–3
H. murinum 1510	2–3	3	0–2	.....	0	0	0	2–3
H. murinum 1548	3–4	4	3–4	3–4	0	0–1	0	0
H. nodosum 1435	.....	0	0	0	0	.....	.....	.....
H. nodosum 1509	0	.....	0	.....	0	3–4	0	3–4
H. pusillum 1650	3–4	.....	0	.....	0	2	3	3–4
Hystrix patula 1477	0–2	0	3–4	3	4	.....	0–1	0

one pot each of *Bromus ciliatus* and *B. purgans;* two other pots of these grasses also were inoculated with aeciospores from *T. dioicum.* Uredia were abundant in both cases. Telia, however, did not appear, until early July

on the grasses inoculated with aeciospores from *T. dioicum*, and about 2 weeks later on the grasses inoculated with aeciospores from *C. virginiana*. Several mounts of the first telia contained the multicellular teliospores characteristic of *P. tomipara;* occasionally a two-cell spore could be found. The telia resulting from aeciospores from *C. virginiana*, however, were all bicellular; the culture clearly belonged to *P. rubigo-vera*, and was designated as culture 23.

It seemed desirable to compare the host range of this Bromus culture of *Puccinia rubigo-vera* with that of *P. tomipara*. Essentially the same series of Bromus species was inoculated with a uredial culture of *P. rubigo-vera* as was used for a similar study of *P. tomipara*.

Inoculations of a series of Bromus species have given the following results:

DISTINCTLY SUSCEPTIBLE: *Bromus purgans* (1289), *B. ciliatus* (1649), *B. altissimus* (1795).

DISTINCTLY RESISTANT: *Bromus adoensis* Höchst. (1331, 815), *B. arduensis* Dum. (1332), *B. arvensis* L. (708, 1297), *B. brizaeformis* F. and M. (1442), *B. commutatus* Schrad. (716, 771), *B. hordeaceus* L. (719, 1508), *B. japonicus* Thumb. (1347), *B. kalmii* Gray. (1541), *B. macrostachys* Desf. (797, 714, 705, 1446), *B. madritensis* L. (713), *B. maximus* Desf. (1427), *B. rigidus* Roth. (1384, 1452, 1501, 698, 1505), *B. rigidus* var. *gussonii* (Parl.) C. and D. (1506, 1333), *B. rubens* L. (1502), *B. sterilis* L. (701, 1503), *B. squarrosus* L. (1450), *B. tectorum* L. (1547, 768, 1545, 712), *B. uniloides* (Willd.) H.B.K. (1507, 1451, 718, 750).

Comparative Morphology of the Urediospores and Teliospores of Eight Cultures of Puccinia rubigo-vera. Attempts to discover morphological differences between races or between physiologic forms within composite rust species have been made by few investigators. Levine (19) has shown that there are significant morphological differences between the spore forms of the 5 races of *Puccinia graminis* that he studied and that these races can, therefore, be distinguished from one another if a sufficiently large number of spores are measured. The same author (20) later showed that there are also slight but constant differences between the spore forms of different physiologic forms within the race *tritici* of *P. graminis*.

Mains (24) noted a certain amount of variation in the size of the urediospores and teliospores of some races of *Puccinia rubigo-vera*. In some cases such variation is quite marked, and this fact accounts for the tendency to separate certain groups of races as species. Mains (24), however, studied spore sizes of most of the races of *P. rubigo-vera* and stated that many races with aecia on Thalictrum did not differ with respect to urediospore size from the races on Impatiens, but that the latter are somewhat outstanding, as a group, for small size of the teliospores. He showed further that cer-

tain races having aecia on a variety of hosts, do not differ to any appreciable extent in teliospore size, but that a few other races with different aecial hosts do show appreciable differences. In general it was concluded that there is too much intergradation of the races of this rust to permit their being distinguished morphologically with any degree of certainty.

In connection with the physiological studies of the cultures of *Puccinia rubigo-vera* used in the present investigation a study of the comparative morphology of the urediospores and teliospores also was made. Only spores produced in the greenhouse, on the most susceptible hosts, and under uniform favorable cultural conditions were used.

For this study of the comparative morphology of 8 cultures of *Puccinia rubigo-vera*, the urediospores, being so nearly globoid, were measured for diameter only. The teliospores, of course, were measured in two dimensions. The length of the teliospore was considered to be that distance from the distal end to the septum separating the spore from the pedicel; the widest part was assumed to represent the diameter of the spore, exclusive of the thickened apex, which is sometimes wider than any other portion. One hundred and fifty urediospores and the same number of teliospores of each rust culture were measured. These were selected at random from a mount in lactic acid, (to render the germ pores more distinct and also to swell the spores to normal size). Only unquestionably mature spores were measured, the measurements being made to the nearest micron.

The data obtained from these measurements are presented in tables 3 to 5. They show rather marked differences between some of the cultures, especially if one compares their mean measurements. Table 3 shows the results obtained from the measurements of the urediospores of the 8 cultures. The range in diameter for the species as a whole, as represented by

TABLE 3.—*Frequencies of the diameters of urediospores of 8 cultures of Puccinia rubigo-vera*[a]

Culture	Classes according to spore diameter									Mean diameter
	15 μ	16 μ	17 μ	18 μ	19 μ	20 μ	21 μ	22 μ	23 μ	μ
1				2	2	40	47	57	2	21.07
8				2	1	38	58	42	9	19.90
11			6	72	51	21				18.58
13			2	25	30	60	24	9		19.70
22	2	16	83	41	13					17.21
14	7	16	74	48	5					17.18
18			1	14	19	92	21	3		19.84
21				42	40	62	2	2		18.94

[a] One hundred and fifty spores of each culture were measured.

TABLE 4.—*Frequencies of the diameters of the teliospores of 8 cultures of Puccinia rubigo-vera*[a]

Culture	Classes according to spore diameter														Mean diameter
	8 μ	9 μ	10 μ	11 μ	12 μ	13 μ	14 μ	15 μ	16 μ	17 μ	18 μ	19 μ	20μ	21 μ	μ
1			1	2	12	16	39	34	15	15	2	10	4		14.94
8			1	0	9	8	44	36	23	20	2	5	2		14.98
11					2	5	28	53	25	13	13	8	3		15.62
13				4	22	18	45	27	20	7	3	2	1	1	13.26
22			3	5	33	28	38	23	7	7	2	3	1		13.77
14			3	2	26	24	45	25	13	7	2	2	1		14.01
18	12	19	36	33	31	13	5	1							10.77
21	14	15	50	26	25	14	4	2							10.67

[a] One hundred and fifty spores of each culture were measured.

these 8 cultures is 15–23 μ; yet only 2 of the cultures (14 and 22) produced any spores that fell within the 15 μ class and only 2 (1 and 8) had any within the 23 μ class, insofar as this could be determined by measuring 150 spores. The modes for urediospore diameter vary from 17 μ, for cultures 14 and 22, to 22 μ for culture 1. The data (Table 3) show a similar difference in the mean diameters of the urediospores of various cultures. Culture 14 had the smallest mean diameter of urediospores, 17.18 μ. Culture 22 is scarcely larger, with a mean diameter of 17.21 μ. There is no correlation here of small urediospore size and aecial host, for 14 goes to Impatiens and 22 to *Clematis virginiana*. Two other cultures (18 and 21), having Impatiens for aecial hosts, have fairly large urediospores, with mean diameters of 19.84 μ and 18.94 μ respectively. The culture having the largest urediospores is number 1 (*Thalictrum* spp. aecial host), with a mean diameter of 21.07 μ.

The teliospores of these 8 cultures also showed differences. In table 4 it will be noted that, for the species as a whole, the teliospores range in diameter from 8 to 21 μ, the extremes being poorly represented. Two cultures are outstanding for narrow teliospores, culture 18 with a mean diameter of 10.77 μ, and culture 21 with a mean diameter of 10.67 μ. The teliospores with the largest mean diameter were obtained in culture 11, which was outstanding for scant and tardy production of teliospores.

The lengths of the teliospores of these cultures ranged from 24 to 53 μ (Table 5). Culture 21 is outstanding for short teliospores, having a mean length of 32.80 μ. Culture 1 was the largest, with a mean of 39.42 μ.

These data show that there is not necessarily a correlation between relative sizes of urediospores and teliospores. For instance, culture 11 has the widest teliospores of the 8 cultures, but the mean diameter of its urediospores is comparatively quite small. Teliospores of culture 18 have a comparatively small mean diameter, but the urediospores of this same culture are fairly large. However, culture 1 has both the largest urediospores and the largest teliospores of the 8 cultures.

Morphologically, a few of the cultures showed some differences. Both 22 and 14 could be separated from the remainder of the cultures because of their small urediospores (Fig. 1), but not from each other. Culture 21 is separable from the others because it has the smallest teliospores (Fig. 2). Culture 18 is separable from the rest by its teliospores of distinctive shape (Fig. 2), which are mostly narrowly wedge-shape and, therefore, differ from the teliospores of the other cultures.

Cultural Experiments with Puccinia tomipara

Culture 20. On Bromus ciliatus and Thalictrum dioicum. Culture 20 originated from aecia collected on *Thalictrum dioicum* near Ann Arbor,

TABLE 5.—*Frequencies of the lengths of the teliospores of 8 cultures of Puccinia rubigo-vera*[a]

Culture	Classes according to spore length															Mean length
	24–25 μ	26–27 μ	28–29 μ	30–31 μ	32–33 μ	34–35 μ	36–37 μ	38–39 μ	40–41 μ	42–43 μ	44–45 μ	46–47 μ	48–49 μ	50–51 μ	52–53 μ	μ
1		2	2	5	14	24	35	18	16	15	4	8	4	2	1	39.42
8			3	9	16	17	40	18	17	17	5	1	5	2		38.75
11			5	12	14	26	44	15	22	9	2	0	1			37.03
13	2	6	7	13	18	22	39	11	14	4	4	7	3			35.96
22		1	2	10	14	11	32	16	12	20	11	8	6	4	3	39.10
14			6	13	14	30	33	22	14	6	6	4	1	1		37.85
18	2	6	9	14	15	11	27	27	18	14	4	3				36.85
21	2	9	15	26	35	28	19	11	1	4						32.80

[a] One hundred and fifty spores of each culture were measured.

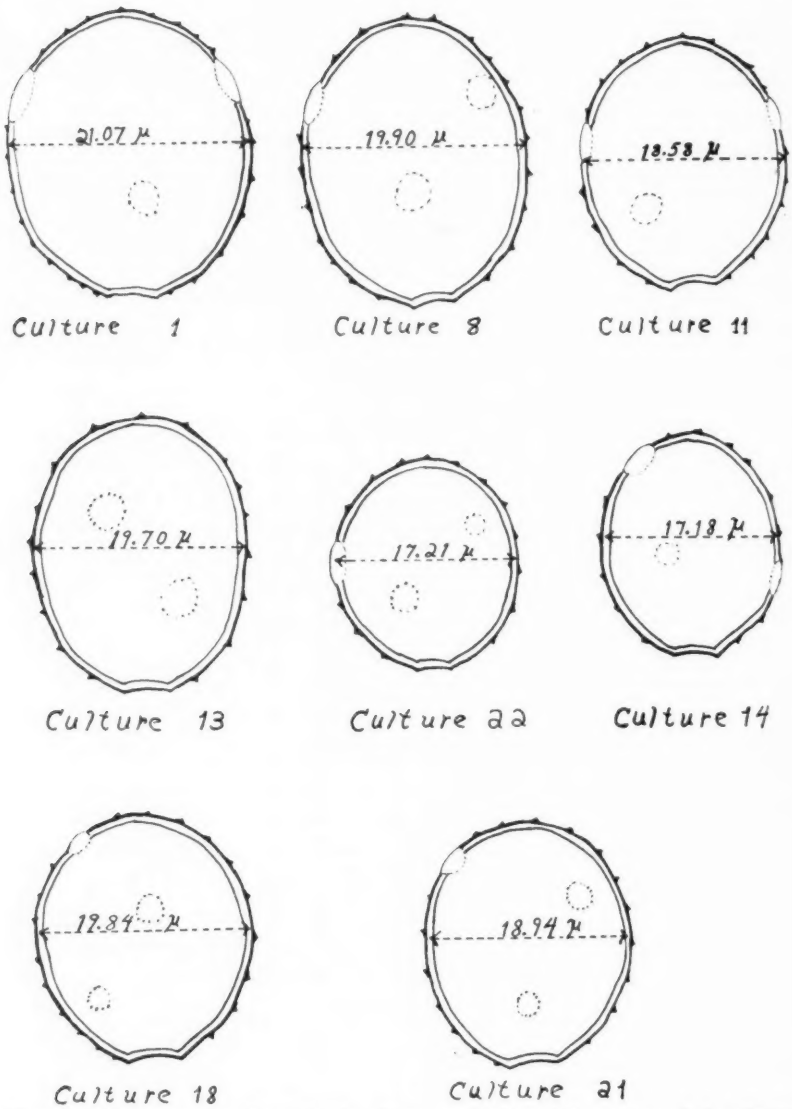

FIG. 1. Diagrams (median view) of representative teliospores of 8 cultures of *Puccinia rubigo-vera* to show differences in the mean dimensions of the spores of each culture.

May 30, 1932. In close association with these aecia were the weathered remains of a species of Bromus, identified the following autumn as *B. ciliatus*. On the over-wintered leaves and sheaths from the previous season numerous telia of the leaf rust type were found. The teliospores were found to be multicellular and the rust was therefore, *Puccinia tomipara*.

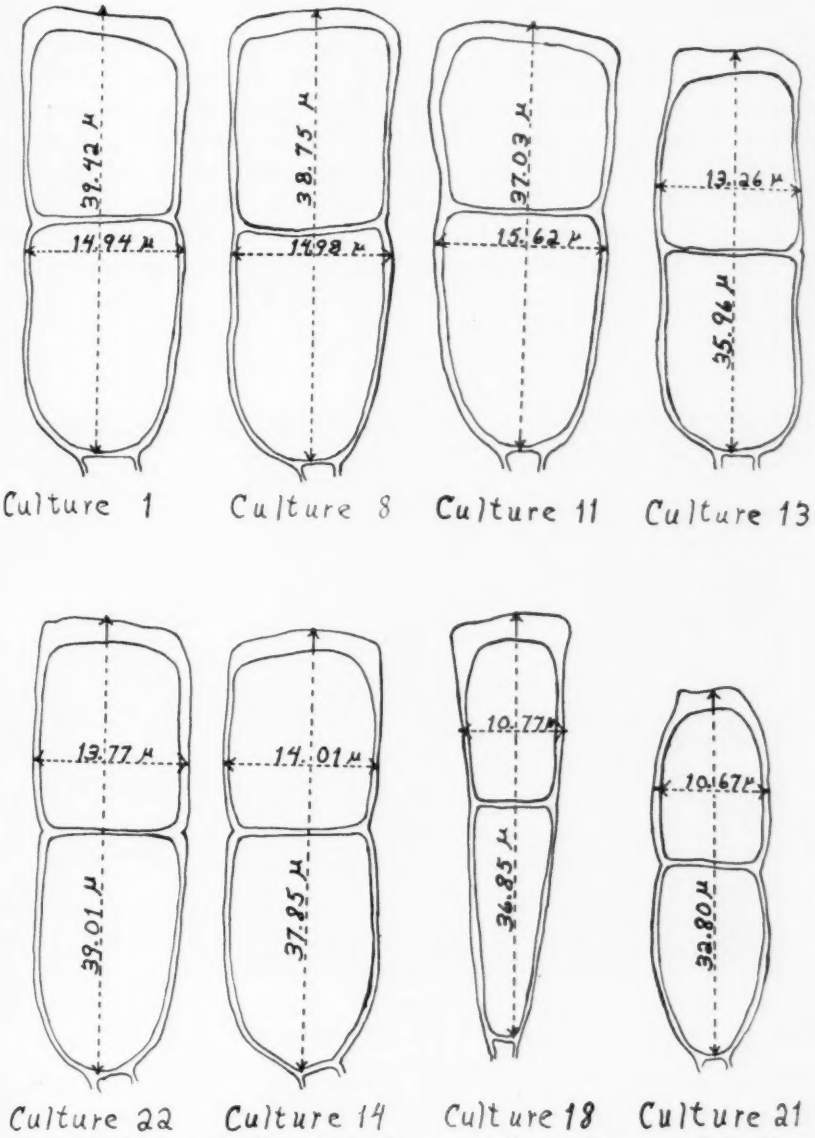

FIG. 2. Diagrams (median view) of representative urediospores of 8 cultures of *Puccinia rubigo-vera* to show differences in the mean diameters of the spores of each culture.

The aecia were used to inoculate several species of Bromus in an attempt to find a favorable grass host. Uredia appeared on *B. purgans* only. This uredial culture was multiplied from time to time throughout the summer. Early in July telia began to appear; these continued to appear

in gradually increasing amounts, so that by the end of November only telia were produced. Examination showed that about 95 per cent of the spores of each telium were multicellular and many were muriform. The telia thus produced in the greenhouse from the original aecial collection, were overwintered in the usual manner. They were germinative even as early as March 8, 1933. A potted plant of *Thalictrum dasycarpum* was inoculated on March 9, resulting in pycnia on March 21 and open aecia on March 31. Seedlings of *B. ciliatus* (1649) and *B. purgans* (1289) were inoculated from these aecia, resulting in a heavy infection on both grasses. The culture was propagated in the usual manner. On May 17, the first telia were collected. Ninety to 95 per cent of the spores of these telia were multicellular, many of them consisting of 6 to 8 cells (Fig. 3). The infection of *T. dioicum* and

FIG. 3. Vertical section through a telium of *Puccinia tomipara* showing the multicellular structure of the teliospores. ×600. (Photograph by E. B. Mains.)

T. dasycarpum was repeated several times. Grass inoculations with aecia collected in the field on *T. dioicum* and *T. dasycarpum* have shown that aecia of *Puccinia tomipara* and *P. rubigo-vera* (see culture 13) may occur side by side on leaves of the same plant and, no doubt, on the same leaf.

Other species of Thalictrum were tested for susceptibility to *Puccinia tomipara*. Inoculations of *T. fendleri*, *T. flavum*, and *T. glaucum* resulted in the production of pycnia and aecia on each.

The host range of *Puccinia tomipara* within the genus Bromus was studied in connection with a similar study of the Bromus culture (Culture

23) of *P. rubigo-vera*. The writer has observed both species present on the same grass hosts in the field, sometimes even intermixing on these grasses, as indicated above (Culture 23). *Puccinia tomipara* has been found in the field on *Bromus ciliatus* and *B. altissimus*.

Inoculations of a series of *Bromus* species with *Puccinia tomipara* have given the following results:

DISTINCTLY SUSCEPTIBLE: *Bromus altissimus* (1795), *B. ciliatus* (1649), *B. purgans* (1289).

MODERATELY SUSCEPTIBLE: *Bromus rigidus* (1384, 1452), *B. rubens* (709, 1502).

DISTINCTLY RESISTANT: *Bromus adoensis* (1441, 1331, 815), *B. arduensis* (1332), *B. arvensis* (708, 1297), *B. brizaeformis* (1442), *B. commutatus* (716, 771, 1349, 1449, 1298), *B. hordeaceus* (719, 1508), *B. intermedius* Gus. (1285), *B. japonicus* (1347, 1551), *B. kalmii* (1541), *B. lanuginosus* (1550), *B. macrostachys* (797, 714, 705, 1446), *B. madritensis* (713, 1362, 1385, 1504, 1447), *B. maximus* (1427), *B. racemosus* L. (711), *B. rigidus* (1481, 1501, 698, 1505), *B. rigidus gussonii* (1506, 1333), *B. secalinus* L. (707), *B. sterilis* (1503), *B. squarrosus* (1450), *B. tectorum* (1547, 768, 1545, 712), *B. uniloides* (1507, 1451, 718, 750).

The specialization of this rust to such a few species of Bromus is interesting. Of 26 species of Bromus inoculated, only 3 closely related species are favorable hosts. Another fact to be noted is the lack of marked reactional differences between different collections within grass species. Only one species (*B. rigidus*) evinced any such differences.

DISCUSSION

It is shown that the multicellular character of the teliospores of *Puccinia tomipara* is constant. No reduction in the percentage of multicellular spores produced was observed while the rust was carried in culture from aecia to telia, back to aecia and then to telia again. Approximately 95 per cent of the teliospores of the telia ultimately produced from the second generation of aecia were multicellular. It is concluded, therefore, that this rust deserves specific rank.

Nine cultures of *Puccinia rubigo-vera* on wild grasses have been shown, in the experimental data presented, to be physiologically distinct. The distinctions have been determined on the basis of the infection type of 8 of the cultures on seedlings of 46 collections of wild grasses of the genera Agropyron, Elymus, Hordeum, and Hystrix, and of one (Culture 23) on species of Bromus. The specialization of the cultures to their aecial hosts serves further to distinguish some of the cultures. Two cultures (22 and 23) produce aecia on *Clematis virginiana;* three (14, 18, and 21) on *Impatiens biflora,* one (1) on exotic species of Thalictrum and one (13) on two native

species of Thalictrum; the aecial hosts for two (8 and 11) were not determined.

According to the concept employed by some investigators (Eriksson (8), Klebahn (17), Arthur and Fromme (4), Arthur (5), and others who have emphasized specialization to aecial host), a few of these cultures could be considered separate species. Culture 14, with aecia on *Impatiens biflora*, comes within the concept of *Dicaeoma impatientis* (Schw.) Arthur, (*Puccinia impatientis* (Schw.) Arth.) as given by Arthur and Fromme in North American Flora (4). The other two cultures (18 and 21), bearing aecia on Impatiens have urediospores somewhat larger than those described for that species. However, all three of these cultures having Impatiens for the aecial host conform well to the variety *P. rubigo-vera impatientis* (Arth.) Mains, as delimited by Arthur (5). Cultures 1 and 22, with aecia on Thalictrum and Clematis, respectively, agree with *D. clematidis* (DC.) Arth. as delimited by Arthur and Fromme (4) in North American Flora, except that the urediospores of culture 22 are too small. Culture 13, with aecia on Thalictrum, also might be placed in *D. Clematidis*, except in this case the teliospores are too small. This would indicate insufficient correlation between spore morphology of the leaf rust of the wild grasses and specialization to aecial hosts for a logical separation of species within the group, thus conforming to the conclusions of Mains (24), who recognized the whole group as an aggregate species, *P. rubigo-vera*, and erected 56 physiologic races, based on culture work of his own and that of previous investigators. Recently, Arthur (5) has recognized 6 varieties within the aggregate species, *P. rubigo-vera*, based chiefly on aecial- and telial-host restriction. Four of these, *viz.*, *P. rubigo-vera tritici* (Eriks. and Henn.) Carl., *P. rubigo-vera secalis* (Eriks.) Carl., *P. rubigo-vera apocrypta* (Ellis and Tr.) Arth., and *P. rubigo-vera impatientis* (Arth.) Mains, are reasonably distinct because the aecia of each are restricted to a different family. In one case (the variety *impatientis*) this distinction is aided by the comparatively small size of the teliospores. The other two varieties, *P. rubigo-vera Agropyri*[4] Eriks.) Arth., and *P. rubigo-vera agropyrina* (Eriks.) Arth. have aecia on hosts of the same family (Ranunculaceae) and have essentially similar grass hosts. They are not morphologically distinct. It would be difficult to assign any of the 3 cultures of this study (nos. 1, 22, 13) having aecia on ranunculaceous hosts to one or the other of these two varieties.

Attempts to identify the 9 cultures of leaf rust of wild grasses with the 56 races of *Puccinia rubigo-vera* described by Mains (24) were unsuccess-

[4] Arthur (5) cites this as "*Puccinia rubigo-vera Agropyri* (Erikss.) n. comb. (*P. agropyri* E. and E)." Since this varietal name apparently applies to the *Puccinia Agropyri* of Ellis and Everhardt rather than to Eriksson's *P. dispersa Agropyri* (*P. agropyrina*) the variety, if maintained, should be designated as *Puccinia rubigo-vera Agropyri* (E. and E.) Arth.

ful. Culture 1, common in Michigan on quack grass, *Agropyron repens*, might be *P. rubigo-vera* sp. f. *persistens* (Plowright) Mains. It, however, differs sharply from that race in that infection was obtained (reaction type 3–4) on strains of *Hordeum jubatum*, *H. pusillum* and *A. tenerum*.

Culture 8 on *Agropyron repens* from Indiana is almost identical in its morphology with culture 1, and, like culture 1, is similar to the race *persistens*. Culture 8, however, differs from this race in its ability to infect strains of *Elymus canadensis*. If either culture 1 or 8 is to be interpreted as belonging to race *persistens*, then the host range of that race must be extended and these 2 cultures interpreted as physiologic forms, differentiated by strains of a few species of grasses.

Culture 11 has been definitely shown to produce a type 3–4 reaction on *Agropyron tenerum*, *A. dasystachyum*, *Elymus canadensis*, *E. glaucus*, *E. robustus*, *E. striatus* var. *arkansanus*, *E. virginicus*, *E. virginicus* var. *glabriflorus*, *E. virginicus* var. *submuticus*, *Hordeum murinum* (1548 only) and *Hystrix patula*. In this combination of hosts the culture approximates *P. rubigo-vera* sp. f. *elymicola* Mains, but on the basis of the results reported by Mains (24) the race *elymicola* does not infect *A. tenerum* or *Hordeum murinum* and does infect *H. gussoneanum*. In a similar manner each of the 9 cultures of *P. rubigo-vera* used in these studies could be shown to differ in grass-host range from any of the 56 races of Mains (24).

Culture 11 might also be considered to be race *septentrionalis* Mains, which Mains (24) based on the studies made by Fraser (11) of a race of *Puccinia clematidis* having aecia on *Actea rubra*. However, definite determination of the race identity of culture 11 can not be made, because Fraser (11) did not inoculate *Agropyron tenerum*, *A. dasystachyum*, *Elymus glaucus*, *E. robustus*, *E. striatus arkansanus* and *Hordeum murinum*.

The fact that none of the 9 cultures can be assigned to any of the races of *Puccinia rubigo-vera* distinguished by previous investigators is due in part to the fact that many of the grasses used by the writer were represented by 2 to several reactionally different collections. The situation in the case of culture 22 may be cited to illustrate this point. This culture bears its aecia on Clematis, and might be expected to conform to one of the 9 races connected with Clematis and recognized by Mains (24). On the basis of the reaction of the grasses he inoculated, culture 22 most closely approximates his race *indianensis*, but apparently cannot be included in this race because *Agropyron caninum* (1466 only), *A. tenerum* (1659) and *Elymus glaucus* (1539 only) proved highly susceptible (resistant to race *indianensis*). Thus it is seen that if the writer's series of grasses had not included those particular strains of these 3 species, culture 22 might well have been considered as *P. rubigo-vera* sp. f. *indianensis* Mains. It might

be considered a physiologic form of this race, distinguished by the reactions of differential strains of *A. caninum*, *A. tenerum*, and *E. glaucus*.

Likewise, the use of more than one collection of the grass species has rendered a few of the cultures separable from the others where such separation would otherwise have been impossible or unsatisfactory. Thus, cultures 1 and 8 would have been considered identical had the series of grasses not chanced to include *Elymus canadensis* (795, 1305, 1366, 1537), *E. glaucus* (1482), *E. virginicus* (1475), *Agropyron repens* (B.G. 13007) and *Hordeum gussoneanum* (1291). The other collections within these same grass species gave identical reactions to cultures 1 and 8. The situation in cultures 14 and 21 is similar, though perhaps less well-marked. These two cultures have the same aecial host (Impatiens) and would have been considered identical had not certain collections of grass species been included in the inoculations, *viz. E. canadensis* (843, 1305, 1366, 1537), *E. virginicus* (765), *A. caninum* (1466), *A. dasystachyum* (1656), *H. gussoneanum* (1421), *H. pusillum* (1650) and *Hystrix patula* (1477).

The manner in which the various strains within the wild grass species differ from each other is similar to the reactional differences that have been found in agronomic varieties of the cereals. The results of these investigations indicate that it would be possible for physiologic forms of the leaf rust of wild grasses to be distinguished one from the other by a standard set of strains or varieties within any one of several species of wild grasses.

The greater the number of strains or differentials used, the greater the possibilities of distinguishing divisions of the rust based on host specialization. If, as these studies indicate, there is in the races of leaf rust on wild grasses, a condition of physiologic specialization comparable to that in the races on the cereals (rye, wheat) almost innumerable forms might be differentiated if variously reacting strains of the wild-grass species are selected and employed.

In previous studies where physiologic forms have been distinguished by strains or agronomic varieties of a host species, only those belonging to one host species have been employed, as, for example, the use of agronomic varieties of wheat to distinguish physiologic forms of *Puccinia rubigo-vera tritici*. Apparently, no attempt has been made to determine the reaction of such physiologic forms on strains or varieties of other species of hosts. It is obvious that such a procedure would soon become very involved and hardly feasible. On the other hand, it is evident that if divisions of species of rusts are made on the basis of specialization to several species of hosts, intraspecific reactional differences should be taken into consideration in order that the results of various investigators may be comparable. As in the study of physiologic forms of the cereal rusts, a standard selected series of strains of the host species involved could be employed.

From the above discussion, the conclusion may be drawn that physiologic specialization in the leaf rust of wild grasses presents a situation quite comparable to that of the leaf rust of cereals and that interpretations of culture work should involve a consideration of the intraspecific reactional differences between collections of wild-grass species.

SUMMARY

Forty-six collections representing 18 species and varieties of wild grasses of the genera Agropyron, Elymus, Hordeum, and Hystrix, were studied with regard to their reaction to 8 cultures of *Puccinia rubigo-vera.*

The reactions of the wild-grass collections to these 8 cultures of leaf rust showed that the cultures are physiologically distinct.

Twenty-six species of Bromus were inoculated with 2 rusts of brome grass, 1 of which was a culture of *Puccinia rubigo-vera,* and the other, *Puccinia tomipara.*

Marked intraspecific reactional differences were noted for various collections of wild-grass species when inoculated with the various cultures of leaf rust.

In view of the fact that strains within wild-grass species may differ markedly from one another with regard to reaction to *Puccinia rubigo-vera,* it becomes evident that if the results of studies of host specialization of this rust are to be comparable, grasses of known genetic constitution should be employed.

Puccinia rubigo-vera on wild grasses presents a degree of physiologic specialization quite comparable to that characteristic of the races of cereal rusts.

Certain cultures of *Puccinia rubigo-vera* differed rather markedly from the others in mean dimensions of the spores. Cultures having the smallest urediospores do not necessarily have the smallest teliospores. Attempts to correlate spore size with host specialization were unsuccessful.

The multicellular character of the teliospores of *Puccinia tomipara* remained constant, through two generations. It is concluded, therefore, that this rust deserves specific rank.

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SEROLOGICAL EVIDENCE IN PLANT-VIRUS CLASSIFICATION

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The discovery that the injection of rabbits with plant-virus extracts induces in such animals the production of antibodies exhibiting various actions on the virus extracts has led to attempts to utilize these reactions in attacking a number of plant-virus problems. Matsumoto and Somazawa (14, 15) have shown that it is possible to follow the invasion of tobacco tissues by tobacco-mosaic virus by means of the precipitin reaction. Recently, Beale (3, 4) has attempted the use of the precipitin test in determining the virus concentrations of tobacco-mosaic extracts. It has been suggested by Birkeland (5) and Beale (3, 4) that the serological reactions might be of use in the identification and classification of plant viruses. The present paper is designed to present a study of this phase of virus serology.

HISTORICAL

Precipitin reactions have been described for tobacco-mosaic extracts by Beale (1, 2) and others and for some constituent or constituents of the potato virus group by Dvorak and others (10). Gratia (11), who first tested 2 different plant-virus sera in the same experiment, showed that tobacco mosaic and a potato virus are not cross-reactive. Beale tested tobacco-mosaic serum against extracts of a number of other plant viruses with negative results, and this finding is suggestive that tobacco-mosaic virus is serologically distinct from these other viruses (1, 2). However, she was unable to perform the reciprocal tests showing that each of these heterologous virus materials was precipitinogenic against its proper serum. Because of the possibility that a negative reaction may be due to a lack of suitable antigen rather than to a lack of close relationship, it is commonly considered necessary in plant serology that all reactions be reciprocally tested before they may be considered as significant in relationship studies (17).

In 1934, Birkeland (5) reported precipitin results with a number of plant-virus antisera, namely sera for tobacco-mosaic, cucumber-mosaic, and "spot-necrosis" viruses. Each virus extract was precipitated by its homologous antiserum, but not by the other 2 heterologous sera. Precipitating sera for tobacco ring spot were not obtained. Birkeland's "ring spot" virus, obtained from Johnson (Johnson's tobacco virus V), is apparently the same

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virus as has been used in the present study and designated as "potato ring spot." This virus has been found by the writer to be serologically very closely related to the latent-mosaic virus of potato (8), and since the "spot necrosis" (Johnson's tobacco virus IV) and the "attenuated spot necrosis" used by Birkeland each reacted uniformly with his "ring spot," one may conclude that all these viruses belong in the latent-potato-mosaic serological group or contain elements of this group. Birkeland's "ring spot," obtained from Henderson, is Wingard's tobacco ring spot, caused by an entirely distinct virus, and the one referred to by the writer in this and other papers as tobacco ring spot.

The writer has shown the antigenic independence of tobacco-mosaic, tobacco-ring-spot, and cucumber-mosaic viruses by means of the specific neutralization reaction. In addition, this reaction has brought evidence of the close relationship of certain symptomatologically distinct strains of each of these viruses (7). In a paper presented at the 1934 meetings of the American Association for the Advancement of Science (8), the writer reported precipitin and complement-fixation reactions indicating a close relationship between a score of strains of tobacco-mosaic virus and between several strains of the latent-potato-mosaic virus, namely the ordinary latent or X-virus, British Queen streak virus, potato-mottle virus, and potato-ring-spot virus. The same paper also indicated the antigenic independence of the viruses of tobacco mosaic, latent mosaic of potato, mild mosaic of potato, aucuba mosaic of potato, cucumber mosaic, and tobacco ring spot. The vein-banding virus of potato was found to react so strongly with cucumber-mosaic virus as to indicate that it is a strain of the latter, and the reactions also suggested that severe etch of tomato was distantly related to tobacco-mosaic virus.

MATERIALS AND METHODS

It is relatively easy to obtain potent antisera to tobacco-mosaic virus and the latent-mosaic virus of potato, but using the techniques heretofore described for preparing sera for these viruses, complete failure was experienced with all of the other viruses mentioned in the present study. The titers obtained, as will be seen below, are not very high, relatively speaking, and other measures must be taken to insure potent immunization.

Virus Stocks

The 20 tobacco-mosaic virus strains used in the present study had been repeatedly purified by the single-lesion method. The strains studied were selected from a larger number of available strains, being chosen because of their striking distinctness from one another and from tobacco mosaic. The sources of the different virus materials were as follows:

Virus	*Source*
Tobacco mosaic and its yellow, white, and slow-moving strains	J. H. Jensen
Masked tobacco mosaic	F. O. Holmes
Latent potato mosaic	E. S. Schultz
Potato ring spot	J. Johnson
British Queen streak	E. S. Schultz
Potato mottle	E. S. Schultz
Mild mosaic of potato	E. S. Schultz
Aucuba mosaic of potato	E. S. Schultz
Vein banding of potato	E. S. Schultz
Severe etch of tomato	W. D. Valleau
Tobacco virus 10729	W. D. Valleau
Cucumber mosaic	R. H. Porter
Tobacco ring spot	S. A. Wingard
Two mechanically transmissible viruses of pea, tentatively referred to as pea-mosaic viruses No. 2 and No. 3	H. T. Osborn

Each of these viruses was tested from time to time for purity, as far as this could be established by inoculations on differential hosts. In the course of the work accidental contaminations were rare and were readily detected and eliminated.

Preparation of Immune Sera

Freshly expressed saps were found preferable to other virus preparations for immunization. With viruses as resistant to physical agencies as those of tobacco mosaic and the latent potato mosaic, expressed saps may be frozen, melted, and centrifuged before using, a procedure that serves to throw down considerable protein and render the extracts relatively clear and nontoxic, although highly infective and antigenic. With other viruses that are more susceptible to destruction on freezing, freshly-expressed centrifuged saps were used exclusively. With moderately resistant viruses, such as those of cucumber mosaic, tobacco ring spot, and aucuba mosaic of potato, intraperitoneal injections suffice, and 5–6 cc. of expressed sap injected in each of 8 inoculations, at intervals of 3–4 days, was the customary dosage. With the less stable viruses, such as severe etch, intraperitoneal injections of freshly-expressed sap proved unsatisfactory, and intravenous inoculations were used, the dosage usually being 2–3 cc. of centrifuged, freshly expressed sap given in each of 8–16 inoculations at 3–4 day intervals. Frequently a combination of these methods (*e.g.*, 4 cc. intraperitoneal + 2 cc. intravenous, per inoculation) proved more satisfactory with such viruses.

The writer has experienced very little loss of animals inoculated intraperitoneally (3 of 67 animals, inoculated intraperitoneally, died during the

period of immunization), but loss from the venous-inoculation method was more considerable (11 of 45 animals inoculated intravenously died during immunization). The animals almost invariably showed a regular and important gain in weight during immunization, this gain averaging 300–500 gm. per month. Accidental death was usually due to violence of reaction to the alkaloids of the inoculated saps, or less frequently to septicaemia or embolism. Rabbit loss has been generally attributed to anaphylaxis in the plant serological literature, but this is not believed to be the usual cause of loss in ordinary work. Various species of Nicotiana, *Solanum tuberosum* L., *Lycopersicon esculentum* Mill., and *Vicia faba* L. were the vehicles of immunization antigens. Of these, the potato proved to be most highly toxic, and satisfactory sera from antigens in this host were best obtained after dialyzing the expressed saps prior to immunizing, a process that removed the toxins without affecting the virus antigenicity. Immediately after venous inoculation with tobacco saps, rabbits usually experience a severe reaction of short duration, marked by spasmodic breathing and convulsions. This lasts but a few minutes, and within a half hour the animals look and act normally.

With viruses that do not immunize readily, a blood probe usually was made from each animal after the 8th and 12th or later inoculations, in order to determine whether or not the animal was sufficiently immune. A sample of 15 cc. of blood was taken 6 days after the last inoculation and following 24 hours of starvation. If the blood reacted unsatisfactorily, inoculations were resumed the following day; if satisfactory, the animal was exsanguinated from the carotid artery 8–10 days after the last inoculation. The blood was allowed to clot for an hour or two at room temperature, centrifuged twice, and the serum was at once frozen until needed for serological testing.

Precipitin Testing

All sera used for precipitin testing were first absorbed by adding 2 parts of expressed sap of the healthy host used in inoculation, and incubating for 2 hours at 37° C. and then overnight in the cold. They were then centrifuged clear and used in testing. Occasionally 3–5 parts of healthy juice were necessary, *e.g.*, with potato, but ordinarily 2 parts were sufficient to eliminate completely any reactions against the proteins normally present in the virus host. It was found necessary for the healthy juice to be in the same condition of preservation as the virus extracts later to be tested.

Precipitin tests were performed in tubes approximately 50×5 mm. (inner diameter), 0.2 cc. each of absorbed serum and virus dilution being pipetted into the tubes and mixed. The pipettes were not tested for inaccuracies in graduation, as the quantitative features of the precipitin reaction are hardly accurate enough to make such a measure of practical

importance. After mixing, a procedure facilitated by the use of a small mechanical shaker, the tubes were incubated at 37° C. for 2 hours and then read; then the precipitate was allowed to settle overnight in the refrigerator. The following morning further readings were made. Only settled flocculations were considered significant and then only when normal serum controls and healthy host-material controls were negative. A single precipitin experiment customarily involved from 100 to 600 tubes.

It is possible to determine precipitin titers either by progressively diluting the serum and testing each dilution against a constant virus extract, or by progressively diluting the extract and testing each dilution against a constant serum dilution. The latter has been found far preferable, for the following reasons: The *occurrence* of a precipitin reaction is independent of the method used, and, within limits, of the non-specific substances incidentally present in serum and sap. Given a positive reaction, however, its *strength* (*i.e.*, the amount of precipitate formed or the dilution to which this can be recognized) is conditioned by the serologically inert proteins also present in the juices. Serum contributes the greater part of all inert protein present in a serum-virus mixture. Hence the progressive dilution of the serum constantly reduces not only the active antibody but the inert substances that give to the virus reaction its volume. Thus progressively diluting the serum, the reaction observed falls short of the titer determined by virus dilution with constant serum, and introduces an added variable. The amount of protein present in plant saps is so inconsiderable, on the other hand, that its dilution, in the presence of undiluted serum, fails appreciably to distort the reaction observed. Therefore, in the present study it has been the practice to hold the serum constant (1:3 to 1:6 of absorbant),[2] and progressively dilute the extracts in a series, 1:1, 1:2, 1:4 . . . 1:512. All dilutions were made in 0.85 per cent NaCl.

The ring test also has been used. The ring test has proved less satisfactory than the flocculation test, however, because of the impossibility of a uniform layering and because frequently the ring titers were lower than those by flocculation.

Test antigens were customarily prepared in the following manner: Expressed saps of healthy and diseased plants were centrifuged, and to 20 parts of each sap was added 1 part of 17 per cent NaCl solution. The saps after shaking were frozen for 24–48 hours, melted, centrifuged, strained through cheesecloth, diluted in series with 0.85 per cent NaCl solution, and tested. Such solutions, if usable, were free from green coloring material and clear. Healthy host controls were prepared in exactly the same manner as virus extracts. It was found to be desirable wherever possible to

[2] As is customary in serological literature, 1:3 is read "one in three," signifying a concentration of 33%.

test the infectivities of test extracts at the time of precipitin testing. This was regularly done in the present study.

Complement Fixation

Beale reported positive complement-fixation tests with tobacco-mosaic materials (1, 2), but her results were not wholly satisfactory because the anti-tobacco-mosaic sera fixed complement so strongly in the presence of healthy-tobacco extract that the only positive observations were of quantitative differences. The following technique gave excellent qualitative results with tobacco-mosaic virus and the latent-potato-mosaic virus, eliminating normal host reactions completely. The sera were prepared from virus materials propagated in tobacco. The test antigens consisted of the same and heterologous viruses propagated in other hosts, *viz.*, pepper, tomato, beet, phlox, and zinnia. No cross-reaction whatever was observed with the healthy host material, and positive reactions occurred with appropriate virus materials in dilutions of 1:1000 or higher. The details of the system used were as follows:

.3 cc. inactive virus-immune serum (1:20) + 1 cc. virus extract (1:1, 1:3, 1:10 . . . 1:1000) + 2 units complement (.2 cc. of 1:10 to 1:20 fresh guinea-pig serum) + 1 hour at 37° C. + .5 cc. sensitized red cells of sheep (= .25 cc. 4 per cent washed sheep cells + .25 cc. inactive anti-sheep amboceptor at 1:1500 + 1 hour at 37° C.) + 1 hour at 37° C. The customary controls were employed for determining the possible hemolytic or anti-complementary actions of virus-immune serum (often hemolytic below 1:20), virus extracts (usually hemolytic or anti-complementary at 1:1, 1:3, and sometimes 1:10, and with tomato at 1:30), and of the other constituents of the reaction. The reactions obtained were very striking, the end points sharp, and the titers high. A zone phenomenon was frequently observed, namely, the inhibition of specific reaction in the presence of excess virus extract, as well as in the absence of sufficient virus extract for maximum reaction.

Although highly satisfactory with tobacco-mosaic and the latent potato viruses, the complement-fixation reaction was negative with tobacco-ring-spot, cucumber mosaic, vein-banding, and potato-aucuba viruses. This is attributed to the fact that, in order for reaction to be error-free, the immune serum and the virus extract must each be diluted to about 1:20. This double dilution lies so far beyond the end point of precipitation with these viruses that even a complement-fixation reaction is not obtained.

"Conglutination" (Mez' Reaction)

Plant-virus materials also exhibit the reaction studied by Mez and termed by him "conglutination"[3] (16, 17). The reaction of Mez was

[3] The term "conglutination" has been used with 3 very different meanings in serology. "Conglutination" was first applied to the normal agglutination of blood corpuscles

utilized in the present study to a very limited extent. The technique, following as closely as possible the directions of Mez, was as follows: To 1-cc. samples of virus extract were added, respectively, .1, .05, .033, .016, .008, and .000 cc. of immune serum. Such mixtures were incubated 1 hour at 37° C. Then to each tube was added .45 cc. of fresh bovine serum ("conglutinin"). The tubes were again incubated at 37° C. and read after 20, 40, 60, 90, 120, and 150 minutes. The use of this technique was restricted to strains of tobacco mosaic.

EXPERIMENTAL

Making use of the techniques described above, the complement-fixation and precipitin techniques have been applied to some 40 strains and distinct types of plant viruses in approximately 10,000 individual tests. A conspectus of the results is given in tables 1 and 2. From these tables the following facts are evident:

1. Tobacco-mosaic virus, the latent-mosaic virus of potato, mild mosaic of potato, aucuba mosaic of potato, vein-banding of potato, tobacco ring spot, and Osborn's pea-mosaic virus No. 2 are all found to be distinct serological entities.

2. Tobacco-mosaic virus, as has been indicated by the studies of Jensen (12), gives rise to many strains, often varying widely in their symptoms. Serologically these strains show such close relationship that they are quantitatively indistinguishable by the precipitin and complement-fixation techniques. The 20 strains tested varied in symptoms on tobacco from a masked strain which caused no symptoms, through the ordinary green mottling field-type of tobacco mosaic, to strains that killed young plants in a short time. The list includes several forms that are locally necrotic on *Nicotiana sylvestris* Spegaz. and Comes, such as the aucuba mosaic of tobacco, although the majority are systemic in this host. It also includes Johnson's yellow tobacco virus VI and certain strains isolated by Jensen, which are characterized by an inordinate slowness in occupying the tissues of the tobacco plant.

3. Such a close serological relationship is shown between the vein-banding virus of potato and cucumber mosaic that one may well be justified in

by such substances as ricin, abrin, and crotin, which, at a relatively high concentration, cause a clumping of the corpuscles. (Miessner and Rewald. Die Konglutination der roten Blutkörperchen durch Ricinussamen. Ztschr. Immunitätsforsch. **2**: 323–349. 1909). Later Bordet, Gay and Streng (Bordet and Gay, Ann. Inst. Pasteur **20**, 467–498. 1906; Bordet and Streng, Centralbl. Bakt. I, **49**: 260–276. 1909) described a reaction designated as "conglutination," in which a substance, "conglutinin," normally present in bovine serum, stimulates complement to function in allowing agglutination and often hemolysis of blood corpuscles by a weak agglutinin normally present in horse serum. The reaction designated by Mez as "conglutination" bears no relation to either of the 2 above, as may be seen from the description of the technique. It is better designated as "Mez' reaction."

TABLE 1.—*Synopsis of precipitin and complement-fixation tests with various plant virus types*

Virus extract:	Immune serum: Tobacco mosaic 9 strains	Tomato severe etch	Potato latent mosaic	Potato mild mosaic	Potato aucuba mosaic	Potato vein banding	Cucumber mosaic	Tobacco ring spot (green)	Tobacco ring spot (yellow)	Osborn's pea virus No. 2	Osborn's pea virus No. 3
Tobacco mosaic (20 strains)	1: 256 1: 1000 +	0 to 1: 32	0 0	0	0 0	0 0	0	0		0	0
Tomato severe etch	0 to 1: 4	1: 16	0		0	0		0	0		
Potato latent mosaic	0 0		1: 64 1: 1000 +		0 0	0 0	0	0		0	0
Potato mottle	0	0	1: 64 1: 480		0	0	0	0	0		
Potato ring spot	0		1: 64 1: 1000	0		0	0				
British Queen streak	0		1: 32 1: 60								
Potato rugose mosaic	0		1: 64			1: 16		0			
Potato mild mosaic	0		0	1: 16	0	0	0	0		0	
Potato aucuba mosaic	0	0	0	0	1: 16	0	0	0		0	
Potato vein banding	0	0	0	0	0	1: 32	1: 16	0		0	0
Cucumber mosaic Valleau's tobacco virus 10729	0	0	0	0	0	1: 16 1: 8	1: 16	0	0	0	0
Tobacco ring spot (green) Tobacco ring spot (yellow)	0	0	0	0	0	0	0	1: 16 1: 32	1: 16 1: 2 to 1: 16	0	0
Osborn's pea virus No. 2	0		0	0	0	0	0	0		1: 8	1: 8
Osborn's pea virus No. 3	0		0	0	0	0	0	0		1: 8	1: 8

Key: Representative precipitin titers not underlined, complement-fixation titers underlined.
Although the table is compiled from numerous experiments, the repetitions have been sufficiently extensive to render the results quantitatively significant to an approximate extent.

TABLE 2.—*Synopsis of precipitin and complement-fixation tests with various strains of tobacco mosaic*

Virus extract:	Immune serum:								
	Tobacco mosaic	Tobacco aucuba	Masked tobacco mosaic	Jensen 101	Jensen 102	Jensen 104	Jensen 108	Jensen 302	Jensen 303
Tobacco mosaic	1: 1000 + / 1: 256	1: 64	1: 100 / 1: 16	1: 30 / 1: 512	1: 96	1: 128	1: 96	1: 30 / 1: 128	1: 128
Tobacco aucuba	1: 128	1: 64	1: 128	1: 256	1: 128	1: 64	1: 64	1: 64	1: 64
Johnson tobacco virus VI	1: 128	1: 64	1: 64	1: 128	1: 64	1: 64	1: 64	1: 64	1: 64
Masked tobacco mosaic	1: 30 / 1: 32	1: 32	1: 100 / 1: 4	1: 30 / 1: 64	1: 16	1: 16	1: 32	1: 30 / 1: 16	1: 64
Jensen tobacco mosaic 101	1: 100 / 1: 128	1: 32	1: 100 / 1: 16	1: 320 / 1: 40	1: 320 / 1: 96	1: 16	1: 320 / 1: 96	1: 320 / 1: 20	1: 320 / 1: 96
Jensen tobacco mosaic 102	1: 48	1: 128	1: 64	1: 320 / 1: 256	1: 320 / 1: 80	1: 64	1: 320 / 1: 48	1: 320 / 1: 20	1: 320 / 1: 48
Jensen tobacco mosaic 104	1: 96	1: 128	1: 64	1: 300 / 1: 128	1: 96	1: 64	1: 96	1: 128	1: 192
Jensen tobacco mosaic 108	1: 128	1: 64	1: 4	1: 64	1: 40 / 1: 16	1: 16	1: 40 / 1: 96	1: 64	1: 40 / 1: 192
Jensen tobacco mosaic 201	1: 256	1: 16	1: 16	1: 16	1: 192	1: 8	1: 96	1: 16	1: 96
Jensen tobacco mosaic 202	1: 192	1: 128	1: 128	1: 256	1: 96	1: 32	1: 96	1: 64	1: 96
Jensen tobacco mosaic 204	1: 32	1: 32	1: 32	1: 32	1: 128	1: 16	1: 32	1: 64	1: 64
Jensen tobacco mosaic 301	1: 64	1: 64	1: 32	1: 64	1: 128	1: 64	1: 64	1: 32	1: 128

TABLE 2.—(*Continued*)

Virus extract:	Immune serum: Tobacco mosaic	Tobacco aucuba	Masked tobacco mosaic	Jensen 101	Jensen 102	Jensen 104	Jensen 108	Jensen 302	Jensen 303
Jensen tobacco mosaic 302	1: 30 / 1: 192	1: 32	1: 100 / 1: 8	1: 30 / 1: 10	1: 40 / 1: 192	1: 16	1: 80 / 1: 96	1: 30 / 1: 10	1: 80 / 1: 96
Jensen tobacco mosaic 303	1: 384	1: 64	1: 32	1: 80 / 1: 20	1: 160 / 1: 192	1: 32	1: 320 / 1: 192	1: 80 / 1: 10	1: 320 / 1: 192
Jensen tobacco mosaic 304	1: 192	1: 16	1: 8	1: 16	1: 192	1: 8	1: 96	1: 8	1: 192
Jensen tobacco mosaic 305	1: 128	1: 128	1: 32	1: 128	1: 128	1: 64	1: 128	1: 64	1: 64
Jensen tobacco mosaic 306	1: 128	1: 128	1: 64	1: 128	1: 96	1: 32	1: 96	1: 128	1: 96
Jensen tobacco mosaic 309	1: 16	1: 16	1: 8	1: 16	1: 32	1: 8	1: 16	1: 16	1: 16
Jensen tobacco mosaic B-7	1: 16	The extracts enumerated to the left all contained appreciable amounts of infective material. Negative precipitin tests were obtained with extracts of the following, which were found to contain little or no infective material: Jensen Nos. 6, 103, 107, 110, 111, 112, 405.							
Jensen tobacco mosaic B-15	1: 32								

Key: Representative precipitin titers not underlined, complement-fixation titers underlined.
As the table was compiled from numerous experiments, the tests obtained are not quantitatively comparable, the significant feature being that all combinations are strongly positive.

regarding these as strains of the same virus type. This heretofore unrecognized relationship is further borne out by the fact that vein-banding and cucumber-mosaic viruses each produce on the cowpea, *Vigna sinensis* (L.) Endl., local brown necrotic lesions that are indistinguishable in appearance. Valleau's tobacco virus 10729 likewise reacted with both vein-banding and cucumber-mosaic sera.

4. Three sera were prepared for severe-etch virus. The first of these reacted positively with tobacco-mosaic virus, the second and third were negative with tobacco-mosaic virus. Tobacco-mosaic serum occasionally showed a weakly positive reaction with severe-etch virus. It is well known that with plant materials high-titered sera often react positively with extracts of distantly related plants, while lower-titered sera have a more restricted specificity. The first of the 3 severe-etch sera had a higher titer than the other 2. Thus a possible but distant relationship is indicated between severe-etch and tobacco-mosaic viruses according to the serological findings. This possible relationship awaits further confirmation from other types of investigation, particularly cross-immunization tests. If substantiated in other ways, the relationship is of particular interest, since the thermal inactivation points, one of the few seemingly solid bases of virus classification, are here very different.

5. The latent-mosaic virus of potato is representative of a group of closely related strains at present designated by such names as latent or healthy-potato virus, X-virus of potato, potato ring spot, potato mottle, British Queen streak, and spot necrosis. Böhme (6) has shown that a number of virus strains may be derived from the latent-mosaic virus of potato and from the potato-vein-banding virus. These viruses of a given type show little or no serological difference. They all cause systemic necrosis in pepper, a characteristic absent from all of the other viruses considered here, with the exception of potato aucuba mosaic, which causes a much more rapid and different-appearing necrosis of pepper. Accordingly, the serological tests thus far performed indicate that the potato viruses studied may be arranged in the following groupings:

a. *Latent potato-mosaic virus* (= X-virus of potato = healthy-potato virus), including as strains:
   - Potato mottle
   - Potato ring spot
   - British Queen streak
   - Spot necrosis (5)
   - Attenuated spot necrosis (5)

b. *Potato aucuba mosaic.*

c. *Potato mild mosaic.*

d. *Potato vein banding* (= Y-virus), including:

Cucumber mosaic
Tobacco virus 1027.9 (Valleau).

e. *Potato rugose mosaic,* as indicated by its serological reactions and as heretofore recognized, a mixture of a virus of the latent potato-mosaic group and potato-vein-banding virus.

6. Two legume viruses isolated by Osborn and designated here as pea-mosaic viruses No. 2 and No. 3, are serologically distinct from all of the other viruses mentioned above, but behave as 2 strains of the same virus type.

It will be noted that in most cases the reactions have been reciprocally tested. Fulfillment of this requirement is hampered at times by the fact that some of the low-titer sera, *e.g.,* mild-mosaic, cucumber-mosaic, and severe-etch sera, rapidly deteriorate in storage, and that it is these same sera that are most difficult to prepare. Indeed there seems to be some indication that the stability of a virus-immune serum varies directly as the stability of the virus concerned. The idea has been held by some serologists, notably Buchner, that antibodies are merely modified antigens. This view most adequately serves to explain their elaborate specificity. The indication that plant-virus antibodies partake of the degree of stability of their respective viruses is in accord with such a view.

The specificity of the serological reactions has been submitted to test in the identification of numerous unknown viruses. A few examples will suffice to illustrate this point. A single leaf of tobacco infected with a virus producing unusual symptoms was submitted for the purpose of determining whether or not it was tobacco-mosaic virus, as it was believed to have arisen from a tobacco-mosaic transplant. On testing, it was found to react not with tobacco-mosaic serum but with tobacco-ring-spot serum. Subsequent infection tests proved that the virus was actually that of tobacco ring spot. A sample of an unknown virus disease in a leaf of an unknown host was submitted with a sample of healthy host material. Precipitin tests showed within an hour that the virus was tobacco-mosaic virus, and this diagnosis was confirmed 2 days later by infection of *Nicotiana glutinosa.* A sample of potato mottle reacted with both tobacco-mosaic and potato-mottle virus sera. A contamination was suspected; the 2 components, tobacco-mosaic and mottle viruses, were isolated separately from the sample and purified, and thereafter the mottle sample showed only its proper reaction. A tomato-streak virus was submitted for identification. Precipitin tests showed it to be a variant of tobacco mosaic and this was confirmed by subsequent infection tests.

In a similar way, through the courtesy of colleagues, samples of the following viruses were submitted as unknowns and were in each case accurately identified as to group by means of the precipitin reaction: ordinary

tobacco mosaic, Porter's cucumber mosaic, tobacco ring spot, latent mosaic of potato, potato ring spot, potato vein banding, a strain of tobacco mosaic from petunia, and mixtures of tobacco mosaic plus cucumber mosaic and tobacco mosaic plus potato ring spot. Occasionally, in such tests of unknown samples, completely negative results have been obtained, but in no case tested has a virus been identified incorrectly.

Some of the serological reactions reported in tables 1 and 2 are illustrated in figures 1 and 2. Figure 1 illustrates the ring and flocculation

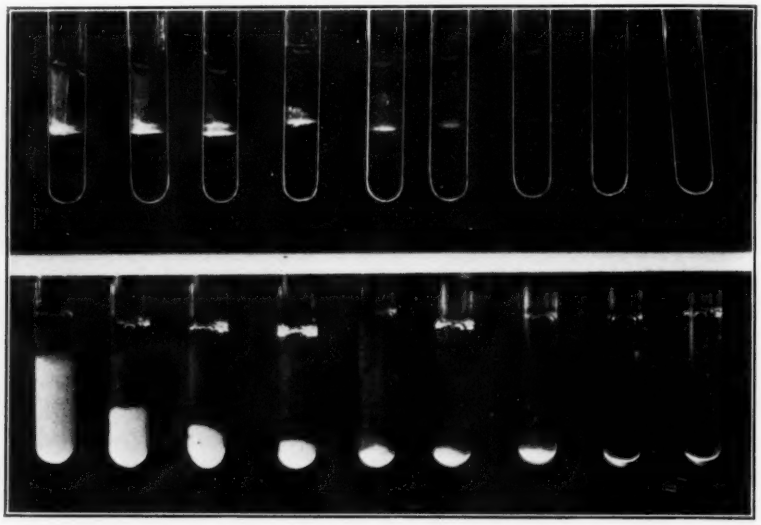

FIG. 1. Ring and flocculation precipitin tests between tobacco-mosaic virus antigen and tobacco-mosaic-immune serum. Explanation in the text.

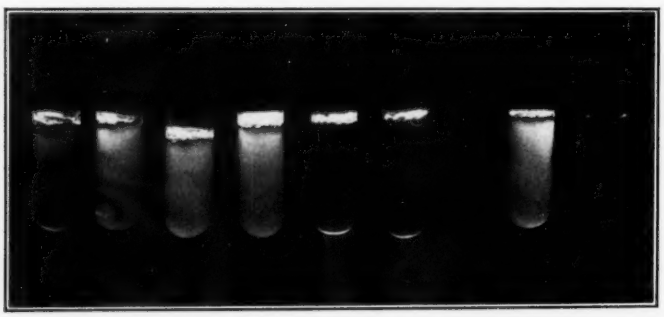

FIG. 2. Complement-fixation reaction between tobacco-mosaic virus antigen and tobacco-mosaic-immune serum. Explanation in the text.

tests applied to the same tobacco-mosaic materials. Each row of tubes from left to right illustrates the results of adding a constant amount of serum to

progressively weaker dilutions of tobacco-mosaic virus extract. The illustration of the ring test (above) shows the reaction as it appeared 1 hour after overlayering serum with virus; the tubes were then shaken, incubated, and the flocculation test is illustrated below as it appeared after further incubation.

A typical complement-fixation test is illustrated in figure 2. The 6 tubes to the left contain progressively-decreasing (left to right) concentrations of tobacco-mosaic antigen in the presence of a constant amount of immune serum and sensitized cells. The 7th tube is a control tube illustrating that the antigen alone (1:3) did not hemolyze nonsensitized cells, and the last tube is a control showing that the antigen alone (1:3) did not inhibit hemolysis. The other controls are not figured. Complete inhibition is seen in the 4th tube (antigen at 1:100), none in the 5th (1:300). The first tube (antigen at 1:3) shows only partial inhibition, a manifestation of the zone phenomenon in the presence of excess antigen.

Mez' reaction ("conglutination") was applied, according to the techniques previously described, to the interreactions of tobacco mosaic and its strains designated by Jensen as 201 and 302. At the same time, ordinary precipitin tests were performed with materials of a similar source. The results obtained showed that, in this case at least, Mez' reaction added nothing to the sensitiveness of the ordinary precipitin test. Difficulty also was experienced with turbidity of the controls. It is concluded that, with the viruses used in the experiments here reported, the precipitin technique is to be preferred, since it avoids the additional chance of error, when in Mez' technique another variable is added (beef serum) to an already complex system.

In conclusion it may be said that serological reactions offer a new criterion in the study of plant virus relationships, a criterion of particular interest and significance because of its subjective character. The writer has shown elsewhere (9) that there is strong evidence that the virus itself is the antigen involved in the reaction. Difficulty has been experienced in the past in deciding on which characteristic of many, stress in classification should be laid. Symptoms alone are insufficient as a criterion. The host ranges of apparently closely related viruses are not always identical. The importance of one criterion and the unimportance of another are largely matters of opinion. In serology we have an arbitrary technique in which opinion has little or no place. It is believed that the viruses are either proteid or that they are living entities, which, like other forms of life, would be basically proteid in constitution. It is probable that in either case a specific protein analysis thus would afford an insight into the basis of their natural classification. Imperfect though they may be, the serological techniques are, at the moment, our chief point of attack in the study

of the chemical basis of specificity (18). In this resides the value of the serological approach to the plant-virus problem, and its empirical results as introduced above support this thesis.

SUMMARY

1. A description is given of techniques that make it possible to prepare immune sera for a considerable number of strains and distinct types of plant viruses. In the present paper the precipitin and the complement-fixation techniques are particularly concerned.

2. The tests show the following virus types to represent distinct serological entities: tobacco mosaic, latent mosaic of potato, potato mild mosaic, potato aucuba mosaic, potato-vein-banding virus, tobacco ring spot, and Osborn's pea-mosaic virus No. 2.

3. From tobacco mosaic have been derived many strains of very close affinity serologically, although of very diverse symptomatology. The group of such uniformly interreactive viruses includes tobacco aucuba mosaic, Johnson's yellow tobacco virus VI, Holmes' symptomless tobacco mosaic, and many of Jensen's brilliant yellow, white, and slow-moving types.

4. The latent-mosaic virus of potato (= X-virus or healthy-potato virus) is representative of a group of serologically closely related virus strains embracing potato mottle, potato ring spot, and British Queen streak. According to Birkeland's tests (5), spot necrosis and attenuated spot necrosis also belong in the same group. Rugose-mosaic virus of potato reacts with both X-virus and vein-banding sera, confirming the present view that it is a mixture of these 2 virus types.

5. The vein-banding virus of potato and cucumber-mosaic virus are so strongly interreactive that these appear to be but strains of the same virus type. This relationship is supported by infection tests. Valleau's tobacco virus 10729 also shows marked affinities with these viruses.

6. A much more distant serological relationship between tobacco mosaic and severe etch of tomato is indicated by precipitin tests.

7. Two viruses isolated from legumes by Osborn and tentatively referred to as pea-mosaic viruses No. 2 and No. 3 behave serologically as strains of the same virus type, although both are serologically distinct from all of the other viruses studied.

8. The usefulness of seric reactions in plant-virus classification has been confirmed by tests of numerous virus samples submitted as unknowns. The results of such tests have shown a sustained accuracy in identification by means of the precipitin test.

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THE ANTIGENICITY OF THE PLANT VIRUSES

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(Accepted for publication April 15, 1935)[1]

Since the discovery that tobacco- and potato-mosaic juices precipitate in the presence of immune sera obtained from rabbits injected with these juices, while after absorption such sera show no reaction against the juices of healthy tobacco and potato plants, considerable emphasis has been laid on the question of whether the virus itself is the active antigen concerned or whether the antigen is an altered host protein, a virus derivative, or a complex of virus and host protein. Beale, Birkeland, and Matsumoto have all expressed the opinion that the active antigen is actually the virus; Beale, chiefly because tobacco-mosaic sera prepared from tobacco material react positively with extracts of several different plants in the Solanaceae, and with extracts of the closely related *Martynia louisiana* Mill. (1, 2) infected with tobacco-mosaic virus; Birkeland and Matsumoto, because their chemically-purified virus preparations showed specific serological activity with virus-immune sera (4, 8). In view of the importance of serological reactions in relation to studies on the nature of viruses and on virus classification, it has seemed desirable to devote some attention to the question of whether the viruses are or are not the active antigens. The present paper reports experiments bearing on this problem. The techniques employed and the virus materials used have been described in previous papers (5, 6).

EXPERIMENTAL

For purposes of orientation, the experiments of Beale and Birkeland mentioned above were repeated and supplemented, with results that may be summarized briefly as follows.

Tobacco-mosaic virus, purified by lead acetate precipitation, retained its antigenic capacity as indicated by the precipitin test. In samples of tobacco-mosaic virus from which more than 95 per cent of the non-virus constituents had been removed, the ratio, virus infectivity: precipitin titer, remained as in the unpurified controls. Purification of tobacco-mosaic virus with silver nitrate gave similar results. Tobacco-mosaic virus when purified by filtration through collodion filters of graded porosity in every case showed a retention of precipitinogenic power in the fraction, filtrate or residue, which retained the infective property, and a loss of both properties in the other fraction. Purification of different viruses by repeated freezing and thawing has likewise given analogous results. It was further found in other preliminary

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experiments that the strength of precipitin reaction varies directly as the amount of infective material in diluted samples of tobacco-mosaic virus, regardless of host species providing the antigenic juice.

PROGRESSIVE INACTIVATION OF PLANT VIRUSES BY VARIOUS MEANS AND RESULTANT EFFECT ON PRECIPITIN REACTIVITY

Certain of the plant viruses that have yielded precipitating sera (6) have thermal inactivation points widely removed from one another. Experiments were performed in order to determine whether or not the seric reactivity of heated virus extracts is lost at the temperature at which each virus is inactivated. The experiments were performed in the following manner.

A frozen stock of virus juice was cleared by centrifuging and distributed in 10-cc. centrifuge tubes in 7-cc. samples. A similar series of healthy host-juice samples was similarly prepared. Retaining unheated a tube of virus and one of healthy juice, tubes of each juice were heated for 10 minutes in a water bath at 5° C. temperature intervals over a range including the reported thermal inactivation point. After cooling the tubes, all were centrifuged and tested at once against absorbed immune and normal sera for the presence of precipitating activity. As soon as possible thereafter an aliquot of each sample was tested on plants suitable for measuring virus activity. The results of the infectivity tests and the precipitin reactions were then plotted on comparable scales, such as are shown in figure 1, which records the results of 4 representative experiments.

The precipitin titers, as given, indicate the highest dilutions to which the heated juices could be subjected and still retain a significant precipitin test (settled flocculation) on the addition of an equal volume of absorbed immune serum at dilution 1:3. The method of determining infectivity, as is indicated in the legend, was adapted to each virus in question.

It is seen from the figure that tobacco-mosaic juice subjected to 85° C. or 90° C. for 10 minutes showed little loss in infectivity, while in samples heated to 95° C. there was a sudden and virtually complete loss of infectivity. The precipitin reactions with the same samples of virus showed no loss in titer up to 85° C., a slight loss at 90° C., and complete loss at 95° C. Analogous results were obtained with the viruses of potato ring spot, tobacco ring spot, and potato vein banding, although in these cases the points at which infectivity and seric reactivity first disappeared were 70°, 65°, and 60° C., respectively.

In all cases there was a heavy protein coagulation at 65° C. This protein coagulation bore no relation to virus inactivation, as is shown by the fact that tobacco-mosaic virus was not inactivated at 70° C. in spite of this coagulation, while the vein-banding virus was completely inactivated at a temperature below the point of heavy coagulation.

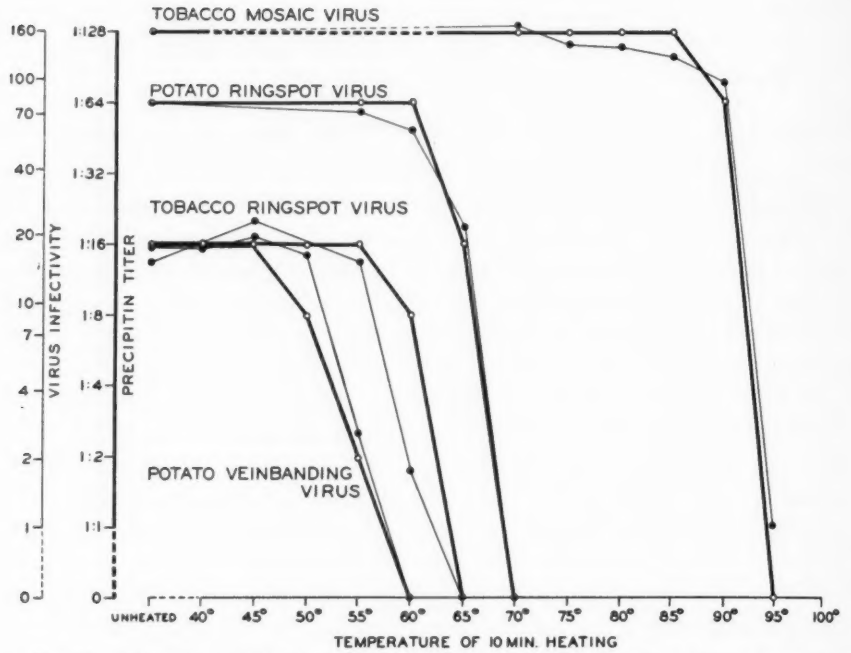

FIG. 1. The effect of 10 minutes' heating on precipitin reactions (coarse lines) and infectivity (fine lines) of certain plant viruses. Infectivity of tobacco-mosaic virus is given in terms of the number of local lesions produced per leaf on 16 leaves of *Nicotiana glutinosa;* that of potato-ring-spot virus in terms of the number of local lesions produced per 2 leaves using 100 leaves of *N. tabacum;* that of tobacco-ring-spot virus in terms of the number of local lesions per leaf on 30 leaves of *Vigna sinensis;* that of potato-veinbanding virus in terms of the number of cases of disease per 50 plants of *N. tabacum.*

All 4 of these viruses had been propagated in *Nicotiana tabacum* L. var. Turkish, and the fact that the seric inactivation curves and the virus inactivation curves nearly coincide for each virus, and that the curves are very different for the 4 viruses used, affords evidence of a dependence of the seric reactions on the presence of active virus, in spite of the common host matrix.

Experiments were also performed in a comparable way to test the effect of inactivation by pH change on the precipitin reaction. In this case, in different experiments, both lead-acetate-purified tobacco-mosaic juice and frozen unpurified tobacco-mosaic juice were used, and both gave essentially similar results. The method of procedure was to divide a virus stock supply into similar samples, to titrate each sample to a given hydrogen-ion concentration, using HCl or KOH, to hold a sample at that pH for a given length of time, usually an hour, and then to neutralize and centrifuge and perform the precipitin and infectivity tests at pH 7.0.

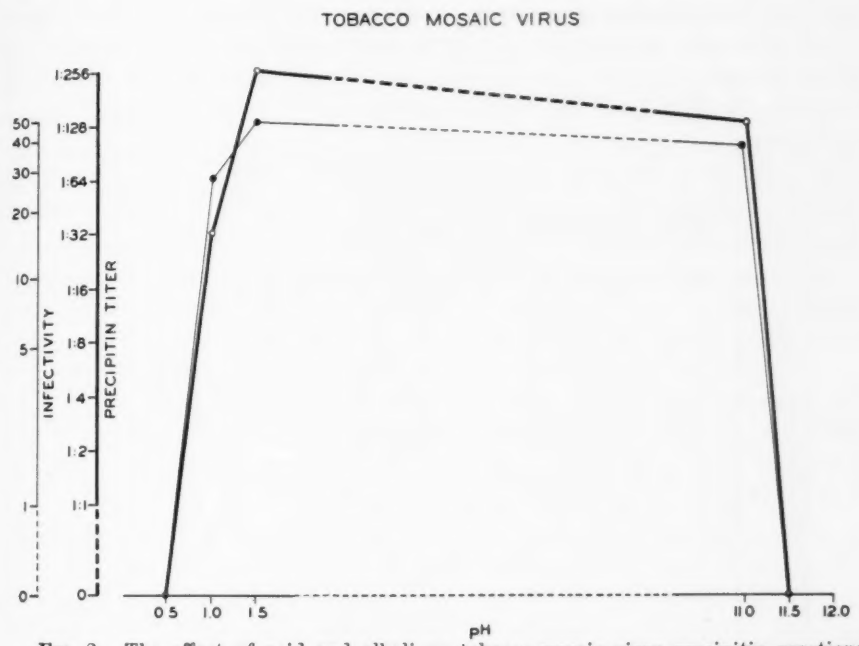

FIG. 2. The effect of acid and alkali on tobacco-mosaic virus precipitin reactions (coarse lines) and infectivity (fine lines). Virus infectivity expressed in terms of the number of local lesions produced per leaf on 16 leaves of *Nicotiana glutinosa*.

Figure 2 illustrates one of several such experiments, all of which gave analogous results. The experiment figured was performed with samples of titrated lead-acetate tobacco-mosaic material for which I am indebted to Dr. W. M. Stanley. From pH 1.5 to pH 11.0 there was no significant loss in either infectivity or precipitin reactivity. At pH 11.5 and pH 0.5 both reactions were completely lost, while at pH 1.0 there was a partial drop in both reactions. The experiment shows that with tobacco-mosaic virus inactivation by pH change the precipitin reaction is retained so long as virus infectivity is retained, and disappears at just the point at which infectivity is lost.

It was next thought advisable to focus attention on chemical virus inactivation, and accordingly a number of experiments were performed in which various viruses were progressively inactivated by chemical treatment and then tested for infectivity and seric reaction. The customary procedure was to divide a stock of frozen virus sap into 5-cc. samples and to add to each sample 5 cc. of reagent, this dilution of the chemical being considered in statements regarding the chemical concentration. After 1–2 hours of incubation at room temperature, each sample was dialyzed against tap water with continuous agitation and using cellophane membranes. After 4–24

hours of dialysis, when the samples no longer gave indication of the presence of the salt used, each sample was centrifuged and tested for precipitin reactivity and infectivity. Healthy host juice was similarly treated as controls. The results of 3 such experiments with tobacco-mosaic virus are plotted in figure 3.

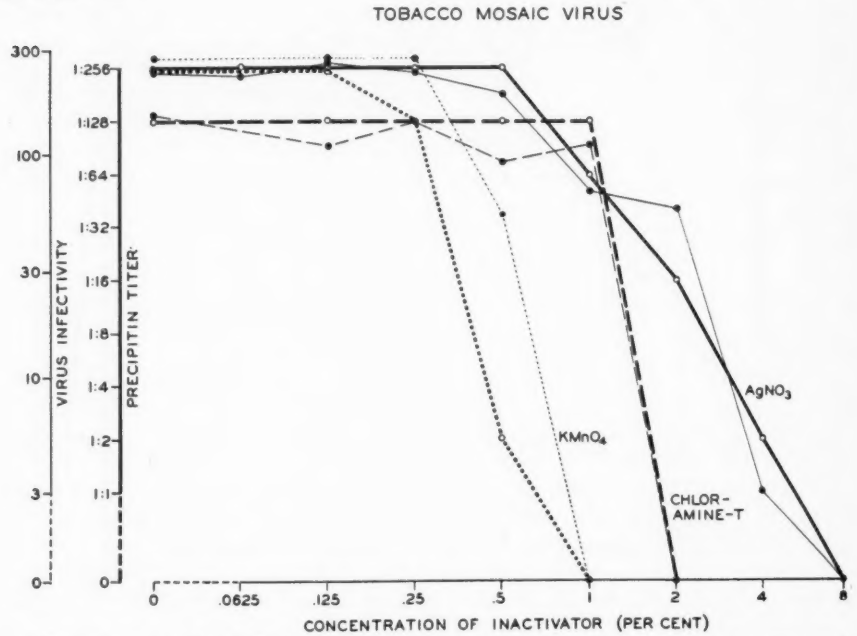

FIG. 3. The effect of certain chemical treatments of tobacco-mosaic virus on its precipitin reactions (coarse lines) and infectivity (fine lines). Infectivity given in terms of the number of local lesions per leaf produced on 16 leaves of *Nicotiana glutinosa* and *Phaseolus vulgaris*.

The figure shows that chloramine-T inactivates all virus at 2 per cent concentration, while practically none is affected at 1 per cent. The precipitin reaction is at its constant maximum with 1 per cent chloramine-T concentration, while at 2 per cent it entirely disappears. Similiarly, $KMnO_4$ eliminates both seric reactivity and infectivity completely at 1 per cent, both partially at .5 per cent, and fails to affect either appreciably at .25 per cent. $AgNO_3$ has a similar effect, the minimum inactivating concentration here being 8 per cent.

The tests with $AgNO_3$ were of special interest. $KMnO_4$ and chloramine-T are both oxidizing agents. $AgNO_3$, on the other hand, like salts of the heavy metals in general, is a powerful precipitant of proteins. In these experiments, in all cases, protein tests were made (trichloracetic acid) and the amount of precipitation produced by the chemical was noted. In the case

of silver nitrate a heavy precipitation of protein took place between salt concentrations .125 per cent and .5 per cent inclusive. The protein precipitated at 8 per cent, 16 per cent, and 32 per cent salt was no greater in apparent amount than at .5 per cent. In spite of this heavy precipitation at .5 per cent, the virus retained practically all of its infectivity and the precipitin test all of its strength. This fact in itself is strong evidence of the antigenic nature of the virus, since nearly all of the protein had been removed at a point at which the virus—notably resistant to ordinary protein precipitants—was neither precipitated nor inactivated, but retained in a pale yellow, water-clear solution.

A very striking phenomenon occurred in the tests with silver nitrate. In performing the precipitin tests with the crystal-clear, pale yellow extracts treated with virus at 0.5, 1, and 2 per cent, the serum precipitate, instead of being white, as in all other virus precipitin experiments heretofore performed, was a golden orange, light at 0.5 per cent, brilliant at 1 and 2 per cent. It is well known that the salts of silver customarily turn yellow. The solutions here tested contained very little protein but an abundance of virus and gave a copious specific yellow precipitin reaction. The yellow color could not have been due to any action of free silver on the serum, since all of the soluble silver that was unattached to protein (or virus) had been dialyzed out, as proved by chemical tests. Moreover, practically all of the non-virus protein had been removed by the silver nitrate precipitation. It also is known that the precipitate in the precipitin reaction contains the virus. It thus seems possible that the silver responsible for the yellow color of the precipitate is attached chemically to the virus forming a yellow soluble virus-silver combination that is infective and specifically precipitated by serum.

Representative experiments showing the effects of different concentrations of $KMnO_4$ and chloramine-T on the potato-ring-spot virus and the potato-vein-banding virus are illustrated in figure 4. In each case the precipitin reaction is retained at full strength as long as the virus infectivity is retained, and the two reactions are lost at the same concentration of chemical, both reactions diminishing at approximately the same rate.

The experiments with chemical treatments thus confirm those with heat, and indicate that the plant viruses studied lose their ability to react serologically at just the point at which they lose their infectivity.

SERIC REACTIONS IN RELATION TO HOST SPECIES SUPPLYING VIRUS SAPS

It has been shown in an earlier paper that each of a number of different plant viruses gives its specific virus seric reaction in spite of the common host matrix (6). The following experiments are representative of a number that were performed in an endeavor to supplement our knowledge of this relationship by testing the seric reactions of the same viruses in different hosts.

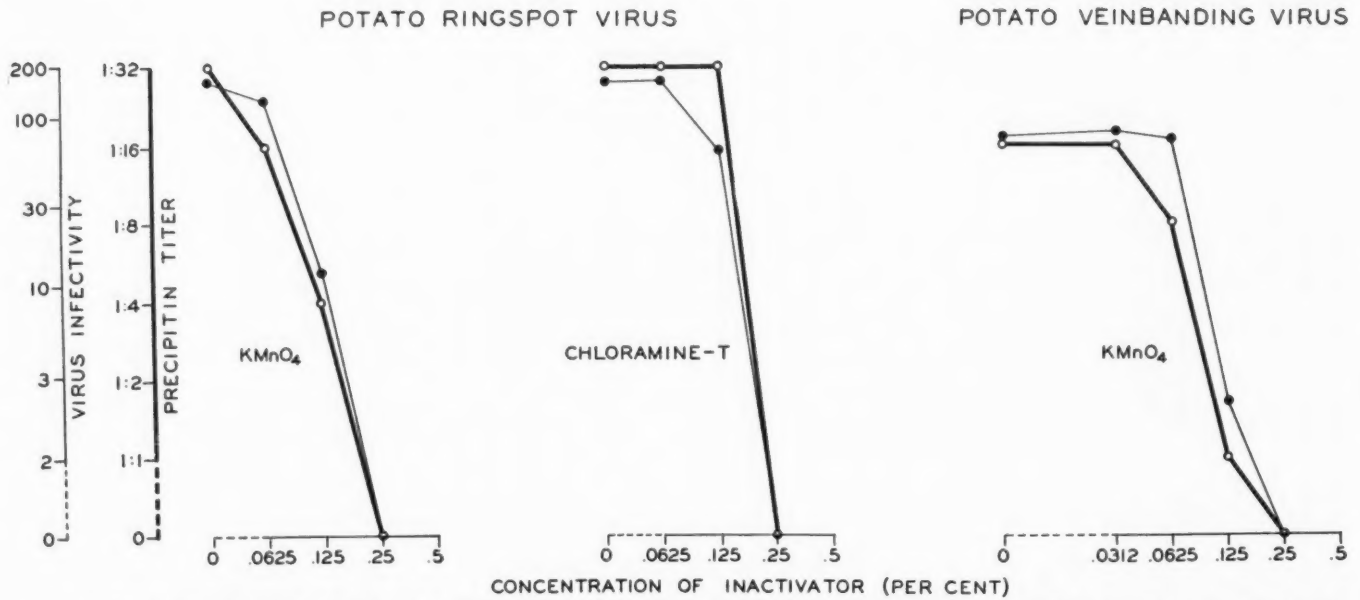

FIG. 4. Effect of certain chemical treatments of the viruses of potato ring spot and potato vein banding on serie reactions (coarse lines) and infectivity (fine lines) of the viruses. Infectivity of potato ring spot given in terms of the number of local lesions produced per leaf on 50 leaves of *Nicotiana tabacum*, of potato vein banding in terms of the number of cases of disease in 50 inoculated plants.

Tobacco-mosaic virus was propagated in tobacco, tomato, petunia, phlox, beet, and zinnia. The expressed saps of these plants were tested serologically against tobacco-mosaic serum prepared from tobacco sap and previously absorbed with healthy tobacco juice. The results are given in table 1.

It is seen from the table that the tobacco-mosaic virus reaction is positive in each case, while the sera are all negative against extracts of these healthy species. Moreover, the virus concentration varies, and is least in beet and zinnia, since the infection here is localized or only slowly systemic. The complement-fixation titers and the precipitin titers of the same extracts show an approximate proportionality to the amount of virus present as given by local-lesion counts. The experiment thus affords evidence that even in species as widely removed from one another as Compositae and Chenopodiaceae, separated in Gray's classification by 21 orders and more than 100 families, positive virus cross-reactions not only occur, but show no significant change in the ratio of precipitin reaction to virus concentration. In species so widely separated, common antigenic proteins do not normally occur.

Incidentally, the same table shows the greater absolute sensitivity of the complement-fixation reaction as compared with the precipitin reaction with the plant viruses. It also brings out the fact that the strengths of reaction by these two methods show similar relative changes from one test antigen to the next. Furthermore, the table illustrates the efficiency of the complement-fixation test by use of the expedient of testing a serum prepared from one host against virus extracts prepared in another host, a technique that eliminates the distorting nonspecific reactions heretofore obtained due to the strong fixation of serum with healthy host material of the same species as was used in serum preparation (1).

Table 2 illustrates an analogous experiment with strains of the potato latent-mosaic virus. The specific immune serum used had been prepared from the virus in tobacco and had been absorbed with healthy tobacco juice. All 4 strains of the latent-mosaic virus when propagated in tomato and the latent-mosaic virus when propagated in potato show strongly positive seric reactions with the absorbed serum prepared from tobacco. Similar experiments have shown that the latent-mosaic virus serum prepared from tobacco reacts specifically with the latent-mosaic virus in pepper, that potato-vein-banding virus serum prepared from tobacco reacts specifically with the vein-banding virus in pepper and potato, that cucumber-mosaic serum prepared from tobacco reacts specifically with cucumber-mosaic virus in zinnia, and that vein-banding and latent-mosaic virus sera prepared from potato react, alone or in mixtures, with their specific viruses in tobacco.

These experiments appear to establish the fact that there is no necessary relationship between host species and virus seric reaction, since, when numerous viruses are propagated in the same host, each gives its specific virus re-

TABLE 1.—*Precipitin and complement-fixation reactions with tobacco-mosaic virus in different hosts*[a]

Antigenic extract in saline dilution series	Concentration of virus[d]	Complement fixation titer[b]		Precipitin titer		
		Tobacco-mosaic serum	Three heterologous virus sera[c]	Tobacco-mosaic serum	Normal serum	Three heterologous virus sera[c]
Tobacco mosaic in tomato (Solanaceae)	150	1:1000 +	0	1:240	0	0
" " " petunia "	100	1:1000 +	...	1:100	0	...
" " " phlox (Polemoniaceae)	100	1:1000 +	...	1:120	0	...
" " " beet (Chenopodiaceae)	10	1:300	...	1:30	0	...
" " " zinnia (Compositae)	30	1:480	...	1:30	0	...
Three heterologous viruses in tomato[c]	....	0	...	.....	0	...
Healthy tomato	....	0	0	.....	0	0
" petunia	....	0	...	.....	0	...
" phlox	....	0	...	.....	0	...
" beet	....	0	...	.....	0	...
" zinnia	....	0	...	.....	0	...
Physiological saline	....	0	0	.....	0	0

[a] All sera prepared from virus material propagated in tobacco, and absorbed to remove precipitins for healthy tobacco proteins.

[b] All necessary controls employed and error-free, but not given in above table.

[c] Latent-mosaic virus of potato, potato-vein-banding virus, potato-aucuba-mosaic virus.

[d] Approximate number of local lesions per *Nicotiana glutinosa* leaf, using 1: 50 virus extract as inoculum.

TABLE 2.—*Precipitin and complement-fixation reactions with the latent-mosaic virus of potato and its strains*[a]

Antigenic extract in saline dilution series	Complement-fixation titer[b]		Precipitin titer		
	Latent-mosaic virus serum	Three heterologous virus sera[c]	Latent-mosaic virus serum	Normal serum	Three heterologous virus sera
Latent-mosaic virus of potato in tomato	1:1000 +	0	1:60	0	0
" " " " " " potato	1:100	...	1:10	0	...
Potato ring spot in tomato	1:1000 +	...	1:60	0	...
Potato mottle " "	1:480	...	1:60	0	...
British Queen streak in tomato	1:60	...	1:30	0	...
Three heterologous viruses in tomato	0	...	0	0	...
Healthy tomato	0	0	0	0	0
Physiological saline	0	0	0	0	0

[a] All sera prepared from virus material propagated in tobacco, and absorbed to remove precipitins for healthy tobacco proteins.

[b] All neccessary controls employed, and error-free, but not given in table.

[c] Tobacco-mosaic virus, potato-vein-banding virus, potato aucuba-mosaic virus.

action, while conversely, when the same virus is propagated in diverse hosts a common virus reaction is retained regardless of host.

DISCUSSION

The evidence thus far adduced regarding the antigenicity of the plant viruses may be briefly summarized as follows:

1. Tobacco-mosaic virus that has been subjected to purification by the use of lead acetate, acetone, trypsin, aluminum hydroxide, barium hydroxide (4, 8), alternate freezing and thawing, and $AgNO_3$ precipitation, retains its ability to react specifically with tobacco-mosaic immune serum prepared either from whole sap or from purified tobacco-mosaic juice. Moreover, the ratio, virus amount: precipitinogenic power, remains constant throughout such purification.

2. Viruses of tobacco mosaic (8), vein banding of potato, latent mosaic of potato, and tobacco ring spot, when subjected to progressive inactivating treatment by heat and by various chemicals, in every case lose their infectivity at just the points where their precipitin reactions are lost, and when approaching these points of complete inactivation their precipitating capacity diminishes at approximately the same rate at which they are being inactivated.

3. Immunization of rabbits with plant-virus extracts produces sera that specifically and quantitatively neutralize the viruses concerned in the presence or absence of precipitin reactions,[2] a result that is interpreted as due to the primary antigenicity of the viruses themselves (5).

4. The ease of immunization of animals and apparently also the stability of the sera (6) vary directly with the stabilities of the viruses used in immunization, as indicated by their resistances to heat and to aging.

5. Fractionating tobacco-mosaic extracts by filtration through graded membranes in each case shows the precipitating power to be retained in the fraction containing the infective material (4).

6. The precipitin and complement-fixation reactions are approximately proportional in strength to the amount of infective material present in diluted virus samples (3).

7. When numerous viruses are propagated in the same host species each gives its specific virus reaction (6), and when the same virus is propagated in species very widely separated taxonomically, the common serological reaction is retained, and, regardless of species, still varies in strength proportionately to the amount of infective material present.

It is concluded that the evidence now available is sufficiently strong to warrant the assumption that the antigens responsible for the plant-virus serie

[2] Chester, K. S. Independence of specific neutralization and precipitin reaction with certain plant viruses. (Unpubl. ms.). 1934.

reactions are the viruses themselves and not normal or derived constituents of diseased plants.

Matsumoto and Somazawa (7, 8) treated tobacco-mosaic extract with formalin and trypsin and found that the precipitin reaction was retained, although infectivity was partially or completely lost. Stanley, however, (9) has shown that inhibitory effect of trypsin, as of many other substances, is an effect on the host plant, not on the virus; and, accordingly, Matsumoto and Somazawa's trypsinized extracts very probably contained much or all of the tobacco-mosaic virus, while their infection tests were negative because of the effect of the reagent on the host plant. Thus this finding, which has been cited as unfavorable to the view that viruses are antigenic, is better interpreted on other grounds.

The fact that inactivated virus is not antigenic might seem to indicate that the viruses are fundamentally different from certain low forms of life. It is well known that dead bacteria retain their antigenic potency and specificity. It is by no means established, however, that the bacterial protoplast is the antigen in the bacterial reactions, and recent work suggests that the peripheral structures of bacteria are serologically important. It is to be expected that these superficial structures have a greater stability than the protoplast within, *i.e.*, that they retain their serological activity after the protoplast is killed. The findings with the plant viruses, on such a basis, offer no grounds for objection to a biotic conception of these agents.

SUMMARY

1. The findings of Beale and Birkeland, respectively, that within the Solanaceae the tobacco-mosaic precipitin reaction is independent of host species, and that purified tobacco-mosaic virus retains its specific precipitin reactivity, are confirmed. The latter finding is extended by the use of additional methods of purification.

2. When tobacco-mosaic, tobacco-ring-spot, potato-vein-banding, and potato-ring-spot viruses are inactivated by series of progressive strengths of chemicals, or by progressive degrees of heating, and when tobacco-mosaic virus is inactivated by progressive changes of pH, or progressively fractionated by filtration, in each case the seric reactions are retained as long as the virus is present and active, they diminish in strength in a way closely parallel to the progressive loss in infectivity, and they disappear at just the point at which the virus becomes no longer demonstrable.

3. When tobacco-mosaic virus and potato latent-mosaic virus-immune sera are tested with their respective viruses propagated in hosts distantly removed systematically from the hosts used in serum preparation, the seric reactions are demonstrable, correlated with the amount of infective material and independent of the host plants used.

4. From these findings, as well as from the facts previously reported concerning virus neutralization, the possibility of arriving at a logical classification of viruses by means of serological reactions, and the correlation between strength of serological reactions and the amount of infective material present in test samples, it is concluded that the evidence now available is sufficiently strong to warrant the assumption that the antigens responsible for the plant-virus serological reactions thus far studied are the viruses themselves and not normal or derived constituents of diseased plants.

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THE EFFECT OF LIGHT ON THE INITIATION OF RUST INFECTION[1]

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Since Hart (2) reported that germ tubes of *Puccinia graminis tritici* Eriks. and Henn. did not enter wheat stomata except while the stomata were open during hours of sunlight and, since Caldwell and Stone (1) reported that germ tubes of *P. triticina* Eriks. seemed able to force entrance through the closed stomata of wheat seedlings, extensive experiments were planned to determine the effect of light during the early stage of the incubation period for several different rusts.

From Loftfield's work (3) and from our own observations it appears that almost all the stomata of cereals are closed in darkness, even in extremely high atmospheric humidity. If the germ tubes of cereal rusts enter their hosts during the hours of darkness it may be assumed that they make their way between the guard cells of closed stomata or that occasional stomata remain open, because they cease to function entirely or are clogged by dust particles, or are situated near the ends of veins and close to the water supply of the leaf. It also is possible that stomata open temporarily in darkness when there is an excess of CO_2 (see Scarth, 5). If germ tubes are able to penetrate closed stomata we might expect almost as great severity of infection when plants are in darkness as occurs under normal conditions of alternating light and darkness with long periods of high atmospheric humidity. If entry is through the occasional stomata that remain open we might expect the severity of infection occurring in darkness to be very much less than normal.

In the following experiments 2 physiologic forms of *P. graminis tritici* were used: form 21 on seedlings of the wheat varieties, Marquis C. I. 3641, Hope C. I. 8178, Acme C. I. 5284, Velvet Don C. I. 1445, Mindum C. I. 5296, Webster C. I. 3780, and Marquillo C. I. 6887, seedlings of the first 5 varieties being completely susceptible to this form while the other 2 are resistant; and form 49 on seedlings of Marquis, Velvet Don, Mindum,

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Hope, and Marquillo, the variety Marquis being susceptible and the others resistant to this form.

Puccinia triticina 53 was used, with seedlings of the completely susceptible Little Club C. I. 4066 wheat as hosts.

Seedlings of Gopher oats C. I. 2027 and Victory oats C. I. 1145 were inoculated with forms 1 and 17 of *Puccinia coronata* Cda. Both varieties are susceptible to both rust forms.

Puccinia sorghi Schw. was included in the experiments and was tested on young plants of the Golden Bantam variety of corn, *Zea mays* L.

In addition, 3 other rusts were used, although we were uncertain as to the distribution of stomata on their host plants and the behavior of the stomata in darkness. Young plants of *Phaseolus vulgaris* L. (Kentucky Wonder beans of commercial source) were inoculated with *Uromyces appendiculatus* Fries; cotyledons and seedling leaves of *Helianthus annuus* L. were inoculated with *Puccinia helianthi* Schw.; and plants from cuttings of *Antirrhinum majus* L. were inoculated with *P. antirrhini* D. and H.

The experiments were made in the winters of 1931–32 and 1932–33. The "light series" was run in the greenhouse at a temperature range of 18° to 25° C., averaging about 21° C. There were about 9 hours of daylight during the winter months, with slightly more in autumn and early spring. Some of the "dark series" were run in a photographic dark room where the temperature usually was 20–22° C. An electric fan circulated the air in the dark room and conditions remained fairly uniform and comparable with those of the light series throughout the experiments. Others of the dark-series experiments were made in a heavy zinc box, $4 \times 4 \times 3$ feet, tightly fastened and padded to keep out light, but with no facilities for circulation of air.

Most of the inoculations were made in the evening after dark, when we might be reasonably certain that the stomata of the cereals were closed. In some cases plants had been in darkness from 1 to 3 hours before they were inoculated; more often 5 to 6 hours elapsed before inoculation; and in some experiments the plants were kept in darkness 8 to 12 hours or longer before inoculation. In one case wheat seedlings were kept in the dark 48 hours previous to, and 56 hours after, inoculation, with no apparent injurious effect on the metabolism of the seedlings. With oats, however, a long period in darkness was detrimental to the development of the seedlings, which were etiolated so severely that some failed to recover when returned to light. By planning rather short periods of darkness previous to inoculation and not too long a period in the moist chamber, it was hoped to avoid the possibility of temporary opening of stomata in darkness that Scarth (5) suggested might take place in *Zebrina pendula* if CO_2 accumulated.

Abundant inoculum was obtained from stock cultures in the greenhouse and was applied to the moistened leaf surfaces by flattened needles or was rubbed on with the fingers. The inoculated plants were kept in moist chambers for varying lengths of time, usually 12 to 24 hours and occasionally as long as 72 hours. For the dark series the plants were in darkness throughout the moist-chamber period, and usually the plant surfaces were allowed to dry before the plants were returned to the light. In the light series conditions were more or less comparable to field conditions, the inoculated plants being placed in moist chambers during the night and then exposed to alternating periods of darkness and light. At the end of the moist-chamber period, plants were returned to the greenhouse bench until infection notes could be taken. In some cases 12 to 14 days elapsed before final notes were taken, but usually 10 days sufficed for the eruption and normal development of rust pustules, yet insufficient for secondary infection. The type of rust infection was recorded, for some hosts were completely susceptible and some were resistant. The prevalence of infection (the percentage of plants infected) was determined in all experiments, allowance being made for seedlings that emerged or leaf blades that expanded after inoculation. Severity of infection (the abundance and distribution of rust uredia over a leaf surface) also was determined according to scales set up for the different rusts, patterned after the scale used by the Division of Cereal Crops and Diseases for estimating rust percentages. Five classes were established, the 3 lowest constituting a light-infection group, the 2 highest forming a heavy-infection group. The average percentages of infection for the 3 lowest classes established were trace, 5 per cent, and 10 per cent. In the other 2 classes severity of infection averaged between 25 and 40 per cent for one class and between 55 and 80 per cent for the other.

In a few experiments attempts were made to study the stomatal movements of the inoculated plants, but removal of plants from moist chambers for stomatal observations usually reduced the relative humidity of the atmosphere and interfered with the proper incubation of the inculated plants.

THE PREVALENCE OF RUST INFECTION IN LIGHT AND IN DARKNESS

In all the experiments accurate counts were made of the number of plants infected and the number inoculated (Table 1). The prevalence of infection of all rusts except *Puccinia graminis* varied from 82.8 per cent to 100 per cent when the plants were incubated in the light. Under the same conditions the prevalence of *P. graminis* was somewhat lower, ranging from 70.0 to 90.1 per cent, but being as low as 54.8 per cent when the resistant Hope wheat was inoculated with form 49. In general, however,

TABLE 1.—*The effect of light during inoculation and the early stages of infection on the prevalence and severity of infaction for seven rust species*

Rust	Host	Light conditions in moist chamber	Prevalence of infection			Severity of infection	
			Number of plants inoculated	Number of plants infected	Percentage	Percentage plants with light infection	Percentage plants with heavy infection
Puccinia triticina	Little Club wheat	Light	157	131	83.4	19.1	64.3
		Dark	389	342	87.9	3.8	84.1
P. antirrhini	*Antirrhinum majus*	Light	782	648	82.8	44.6	38.2
		Dark	392	358	91.3	39.9	51.5
P. coronata	Gopher oats	Light	75	73	97.3	40.0	57.3
		Dark	165	163	98.7	36.3	62.4
"	Victory oats	Light	377	335	89.0	12.8	76.2
		Dark (Short)	135	125	92.6	33.3	59.3
		Dark (Long)	236	134	56.7	56.7	0.0
P. helianthi	*Helianthus annuus*	Light	106	106	100.0	16.0	84.0
		Dark	58	54	93.1	24.1	69.0
P. sorghi	*Zea mays* (Golden Bantam)	Light	436	395	90.6	24.3	66.3
		Dark	231	164	70.9	31.6	39.3
Uromyces appendiculatus	*Phaseolus vulgaris* (Kentucky Wonder)	Light	68	60	88.2	27.9	60.3
		Dark	60	46	76.6	43.3	33.3
P. graminis tritici 21	Marquis wheat	Light	440	341	77.5	6.1	71.4
		Dark	535	281	52.5	40.4	12.1
	Hope	Light	435	348	80.0	5.1	74.9
		Dark	436	256	58.7	28.0	30.7
	Acme	Light	91	71	78.0	8.8	69.2
		Dark	67	42	62.7	38.8	23.9
	Velvet Don	Light	235	169	71.9	4.7	67.2
		Dark	215	116	53.9	20.9	30.0

TABLE 1.—(*Continued*)

Rust	Host	Light conditions in moist chamber	Prevalence of infection			Severity of infection	
			Number of plants inoculated	Number of plants infected	Percentage	Percentage plants with light infection	Percentage plants with heavy infection
P. graminis tritici 21	Mindum	Light	81	73	90.1	0.0	90.1
		Dark	80	60	75.0	16.3	58.7
	Webster	Light	39	34	87.2	53.9	33.3
		Dark	50	17	34.0	34.0	0.0
	Marquillo	Light	313	220	70.2	9.2	61.0
		Dark	324	191	58.9	19.7	39.2
P. graminis tritici 49	Marquis	Light	216	172	79.6	17.6	62.0
		Dark	248	95	38.3	26.2	12.1
	Velvet Don	Light	191	150	78.5	15.7	62.8
		Dark	152	81	53.3	26.3	27.0
	Mindum	Light	209	153	73.2	13.9	59.3
		Dark	232	139	59.9	17.2	46.7
	Hope	Light	135	74	54.8	32.6	22.2
		Dark	147	42	28.5	24.5	4.0
	Marquillo	Light	414	290	70.0	26.1	43.9
		Dark	347	209	60.2	23.9	36.3

with all the rusts concerned, a large proportion of inoculated plants became infected.

When plants were inoculated in darkness and darkness prevailed throughout the moist-chamber period, the prevalence of infection usually differed from that obtained on plants in the light. With *P. graminis tritici* it was considerably less than in light—from 9.8 to 53.1 per cent less, depending upon the rust form and the host. In the variety Marquis, completely susceptible to both rust forms, the reduction in prevalence of infection in darkness was 25.0 per cent with *P. graminis tritici* 21 and 41.3 per cent with form 49. In the resistant Marquillo, on the other hand, the reductions were 11.3 and 9.8 per cent, respectively.

With *Puccinia sorghi* the prevalence of infection in darkness was nearly 20 per cent less than in light, and with *Uromyces appendiculatus* there was a reduction of 11.6 per cent. However, darkness seems not to reduce the prevalence of infection of some other rusts. With *Puccinia triticina* the prevalence of infection was actually slightly higher in darkness than it was in light (4.5 per cent higher), while for *P. antirrhini* the prevalenec of infection in darkness was 8.5 per cent higher than that in light.

In the case of *Puccinia coronata* darkness did not hinder infection, the prevalence of infection on Gopher oats in darkness being approximately the same as in light, and the prevalence on Victory oats being slightly higher in darkness than in light. However, darkness in excess had a decided effect on Victory oats seedlings and the prevalence of crown-rust infection. After 18 to 48 hours in darkness before inoculation and 48 to 60 hours in darkness after inoculation oats seedlings were severely etiolated and on many the tips of the leaf blades were dying. The tissues exposed to rust infection were impoverished, some temporarily, others permanently. The prevalence of crown-rust infection on such plants was reduced to 56.7 per cent, and most of the infections were in the basal region of the leaf blade, the part that recovered first from the great etiolation. The results of these experiments probably are to be attributed to the effect of darkness on host metabolism, not to its effect on stomatal behavior and entry of the parasite.

THE SEVERITY OF RUST INFECTION IN LIGHT AND IN DARKNESS

Perhaps more important than the prevalence of rust infection is the severity of infection: the abundance and distribution of rust pustules over the leaf surface. If the rust inoculum is viable and virulent and has access to its host, it may be expected to cause heavy infection such as is shown in figure 1, A. On Hope and Marquis, the two hosts completely susceptible to form 21 and incubated in the light, the infection centers are numerous and distributed over almost the entire leaf surface and occupy a relatively large proportion of the leaf area. Even on the resistant Mar-

quillo host, in spite of the fact that less of the leaf area seems to be affected, the severity of infection is high, since there are 51 infection centers as compared with 58 and 70 for the 2 completely susceptible hosts. If light has no effect on the entry of rust or the very early stages of infection, we might expect similar severity of infection in light and in darkness. But if the host stomata are closed in darkness and the rust cannot enter its host through closed stomata, we might expect a relatively light infection (Fig. 1, B) or merely a trace of infection. Lack of light reduces the severity of infection by some rusts but has no effect in the case of others.

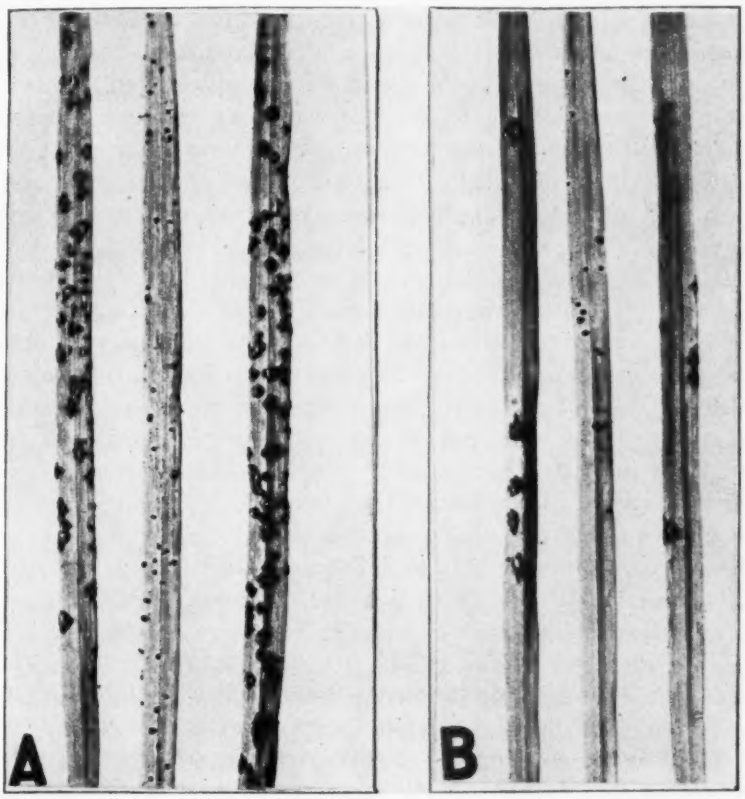

FIG. 1. A. *Puccinia graminis tritici* 21 on seedlings of Hope, Marquillo, and Marquis, inoculated and incubated in the light. There are 58, 51, and 70 infection centers, respectively, even though the resistant host Marquillo has a 2^{+c} type of infection while the susceptible Hope and Marquis have pustules of the 4^{+} type. B. Form 21 on the same varieties, inoculated and incubated in the dark, with 5, 14, and 8 infection centers, respectively.

Most of the seedlings of Little Club wheat rusted by *Puccinia triticina* were severely infected, falling in the 2 highest classes established for infec-

tion severity. Less than 4 per cent of the seedlings that had been inoculated and incubated in the dark escaped with light infection. Thus, as Caldwell and Stone (1) pointed out, darkness during inoculation and throughout the moist-chamber period does not inhibit leaf-rust infection of wheat. The same is true of *P. coronata* on Gopher oats, most of the rusted plants being severely infected whether they were inoculated and incubated in light or in darkness. Some oats plants escaped with light infection, but more than half were severely rusted. Nearly all the wheat plants were severely rusted by *P. triticina*. *Puccinia antirrhini*, the snapdragon rust, also, was not deterred by darkness from infecting its host leaves heavily. As a matter of fact darkness favored severe infection somewhat more than did light. The majority of the plants in darkness were severely rusted, while in light the majority of the plants were lightly rusted.

In the experiments with stem rust of wheat our results are at variance with those of Peterson (4), for in our work darkness reduced the severity of infection in most varieties. As a general rule the majority of plants inoculated and incubated in light were severely rusted, while relatively few were lightly rusted. For example, 71 per cent of the Marquis seedlings inoculated and exposed to light were heavily rusted by *P. graminis tritici* 21 and only 6 per cent were lightly rusted (Table 1). Many of the seedlings of other varieties were rusted as heavily as Marquis was, but there were 2 exceptions: Form 21 produced light infection on the majority of the resistant Webster plants inoculated and incubated in the light; and Hope, resistant to form 49, was more often lightly infected than severely infected when incubated in the light. On susceptible varieties, nevertheless, the majority of plants were severely rusted.

The effect of darkness at inoculation and during the moist-chamber period was to shift the severity of infection towards the lower classes so that the proportion of plants with light infection was increased, sometimes to such an extent that most of the rusted plants were only lightly infected, whereas, in light, the majority had been severely infected. Thus, 40.4 per cent of Marquis seedlings inoculated with form 21 and incubated in the dark were only lightly rusted, while 12.1 per cent were severely rusted (Table 1), thus increasing the percentage of plants with light infection from 6.1 (in light) to 40.4 (in darkness) and decreasing the percentage with heavy infection from 71.4 (in light) to 12.1 (in darkness).

In other cases the shift was not so decided. In the light, Hope infected with form 21 was very much like Marquis—74.9 per cent of the seedlings were severely rusted and only 5.1 per cent lightly rusted. In darkness, however, about half, or slightly more, of the rusted seedlings were severely infected and half lightly infected; that is, 30.7 per cent of the seedlings in darkness were severely rusted as compared with 74.9 per cent severely

rusted in light, while 28.0 per cent in darkness were only lightly rusted as compared with 5.1 per cent in light. In Mindum, on the other hand, when *Puccinia graminis tritici* 49 is used, the change in proportions is very slight. Darkness reduces the percentage of severe infection from 59.3 to 46.7, while the percentage of plants with light infection is increased slightly, from 13.9 to 17.2. The one exception to this general reaction to darkness is found in Marquillo when rusted by *P. graminis tritici* 49. The proportions of lightly rusted and severely rusted plants are approximately the same in light and in darkness.

Darkness had a similar effect on the development of bean rust. Sixty per cent of the bean leaves incubated in the light were severely rusted by *Uromyces appendiculatus,* but in darkness only 33 per cent were severely rusted. The percentage of leaves with light infection was nearly 28 in light but darkness increased this to 43.3 per cent, so that in darkness the majority fell into the light-infection group.

Infections by *Puccinia sorghi* and *P. helianthi* were affected by light, but to a less extent than infections by *P. graminis* and *Uromyces appendiculatus.* Darkness reduced the percentage of plants with severe infection but in no case shifted the severity of infection so much that the majority of the plants were only lightly rusted. Sixty-nine per cent of the sunflowers and 39.3 per cent of the corn plants inoculated and incubated in the dark were severely rusted—and these figures represent majorities of the rusted plants.

Light or its absence did not affect the severity of crown rust on Gopher oats, but on Victory oats darkness slightly reduced the proportion of severely rusted plants and increased the proportion of lightly rusted plants. Darkness in excess resulted in very light rust infection, but, as discussed previously, this was to be attributed to the etiolation and poor vigor of the host.

DISCUSSION

The question of initiation of rust infection during darkness may have a bearing on the opportunities for primary infection and spread of rust in the field, and it may be concerned in the apparent ease with which an outbreak of one rust develops to epidemic proportions while epidemics of other rusts seldom occur or are secured with difficulty by artificial means.

Moisture is the first requisite for spore germination and the growth and survival of germ tubes. If it is the only requisite up to the time the rust sends its haustoria into the host cells, infection might occur whenever moisture was available for short periods—during rains or a series of showers, or when the plants were covered with dew for a few hours. But rust germ tubes enter their graminaceous hosts through the stomata, which

usually are closed in darkness. Some rusts are not impeded by closed stomata, so that moisture still may be considered the prime requisite for the functioning of inoculum and initiation of infection. Other rusts are unable to penetrate closed stomata; consequently there are 2 requisites to be considered: moisture and stomatal openings. Opportunities for infection are somewhat more limited, for it occurs only during the time when there is sufficient moisture and stomata are open. Darkness hinders infection by rusts of this class because most of the stomata close in darkness.

Leaf rust of wheat, crown rust of oats, and snapdragon rust seem to enter their hosts equally well in light or in darkness. On the other hand, darkness hinders greatly the infection of wheat by *Puccinia graminis* and of beans by *Uromyces appendiculatus,* and darkness deters slightly the infection of corn and sunflowers by their respective rusts.

Under some field conditions the restrictive action of darkness may not be apparent and may be of no importance in the initiation of infection and spread of rust. If inoculum is abundant at all times and environment favors continual inoculation and infection, the final amount of rust may be as great and as overwhelming as though darkness had no effect on the entrance of the fungus. But if the inoculum enters an area in waves and environment favors infection only at short intervals during the growing season, the limiting effect of darkness may be sufficient to reduce the amount of infection considerably.

SUMMARY

Experiments were made to determine the effect of light and of darkness on the entry of uredial germ tubes and development of *Puccinia triticina, P. graminis tritici, P. coronata, P. sorghi, P. helianthi, P. antirrhini,* and *Uromyces appendiculatus.*

Darkness at the time of inoculation and throughout the early stages of infection reduced the prevalence and severity of infection by *P. graminis tritici* and *Uromyces appendiculatus.*

Darkness had no effect on the prevalence and severity of infection by *P. triticina* or *P. antirrhini.*

The prevalence of infection by *P. sorghi* and *P. helianthi* was reduced by darkness; and the severity of infection was reduced slightly, but not sufficiently to place the majority of infected plants in the "light infection" class.

Darkness did not affect the prevalence of infection by *P. coronata,* nor the severity of its infection on Gopher oats; but on Victory oats the severity of infection by *P. coronata* was reduced slightly.

The effect of darkness on infection by *P. graminis tritici* varied somewhat according to the wheat variety and rust form used. Infection of the

susceptible variety Marquis was decreased decidedly by darkness, but there was less effect on the infection of the resistant variety Marquillo.

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PHYTOPATHOLOGICAL NOTES

An Internal Necrosis of Bean Seeds.[1]—During the early spring of 1934 some bean seeds were sent to the Department of Biology by W. D. Click, the county agent of Wayne County. These seeds showed brown necrotic spots in the center of the flat inner surface of both cotyledons. There was no external symptom in evidence. These seeds represented a sample taken from a lot that had been purchased for planting by the Relief Administration of Wayne County. They were purchased from H. D. Woodruff and Sons of Orange, Conn., according to advice from Mr. Click. It is not known where they were grown.

Because the disease appeared to be unrecorded in the literature on beans, it seemed advisable to follow it up; consequently a larger sample was secured for planting in the pathologium. No count of germination was taken, but a fair stand was secured and a small crop of pods harvested. At this time it was noted that two varieties[2] were represented in the planting. The broad pod variety was determined as Wooster Mammoth and the narrow pod variety as Jarvis. The markings on the surface of the seeds are similar, but are slightly lighter on Jarvis.

An examination was made of 100 of the harvested pods and the condition of every seed in each pod recorded. The Wooster Mammoth variety showed a very high percentage of this defect. Forty-seven out of 49 pods contained one or more seeds showing the necrotic spot, and in 33 of these pods all seeds were defective. The Jarvis variety appears to be less susceptible. Only 10 pods out of 51 examined showed any defect and in only 2 cases were all seeds in the pod spotted.

Several attempts were made to isolate an organism from the spots. The necrotic area was removed aseptically and placed in sterile broth medium, but no evidence of any associated organism was found. Apparently the disturbance is of a non-parasitic nature and more work is essential before any definite statement can be made.

The only disease known to the writers that bore resemblance to this internal necrosis is the "Marsh Spot" of peas as described by De Bruijn[3] and attributed by her to climatic and soil conditions. Recently, Zaumeyer

[1] Published with the approval of the director, West Virginia Agricultural Experiment Station, as Scientific Paper No. 150.

[2] The pods were submitted to the Bureau of Plant Industry, U. S. Department of Agriculture and identification made by Dr. Victor C. Boswell.

[3] De Bruijn, H. L. G. Kwade harten van de erwten (Marsh Spot of Peas) Tijds. Plantenziek. 39: 281–318, 1933.

FIG. 1. A shows the difference in size of pods and seeds of the two varieties. The larger is the Wooster Mammoth, the smaller the Jarvis. Spotting of the larger variety is very clear. The seeds of the smaller variety show only a very slight defect in one cotyledon, the second from the bottom. B shows several affected seeds. The spots vary in color from a dark brown to a pale yellow and in size from very small markings to discolorations that may cover half the inner surface of the cotyledons. The seed in the center of the figure is healthy.

and Wade[4] have reported a similar internal spotting in western peas and under greenhouse conditions at Rosslyn, Virginia.

In both peas and beans the defect has every appearance of being non-parasitic unless there be a virus concerned. However, it appears that in the case of the bean necrosis there may be heritable tendencies that influence its incidence. Further studies are necessary to clear up these problems.—C. R. ORTON and W. D. HENRY, University of West Virginia, Morgantown, W. Va.

Filtrability of Certain Plant Pathogenic Bacteria.—Phytomonas tumefaciens (Sm. and Town.) Stevens and *Bacterium gypsophilae* Brown are the only phytopathogenic bacteria the filtrability of which seems to have been reported. Rosen[1] observed in young bouillon cultures of *P. tumefaciens* buds that passed through Berkefeld V filters. Lieske[2] reported filtrability of old bouillon cultures of *P. tumefaciens* and not of cultures grown in milk. Schätzel[3] was able to get a filtrable form of *P. tumefaciens* in old as well as in young cultures whether grown in bouillon or milk. Brown[4] established filtrability of *B. gypsophilae* and *P. tumefaciens* when grown in beef bouillon and filtered through Chamberland L_3 filters.

The writer, working on methods of procuring toxins from *Erwinia amylovora* (Burrill) Winsl. *et al.*, observed that some filtrates produced growth in bouillon which appeared to be a pure culture of *E. amylovora.* Investigation, therefore, was undertaken to study filtrability of *E. amylovora.* Tubes of common nutrient broth inoculated with *E. amylovora* were incubated at 28° C. and then filtered through new Berkefeld V- and N and Chamberland L_3 filters at different intervals of time. Filtrates of these cultures tested up to 2 months remained sterile. On the other hand, skimmed milk used as a medium was found to induce a filtrable stage of *E. amylovora.* A large number of tubes of sterile milk inoculated with *E. amylovora* and incubated at 28° C. was filtered and cultured after 7, 12, 24, and 36 days, respectively. The above named filters were used. *Bacillus prodigiosus* (Ehrenb.) Flügge and *Bacterium coli* (Escher.) Lehm. and Neum. always were mixed with the cultures to be filtered to check the efficiency of the filters used. After filtration 1 cc. of the filtrate was transferred into nutrient broth of pH 6.9 and incubated at 28° C. At the same

[4] Zaumeyer, W. J., and B. L. Wade. Physiological spotting of pea seed. Phytopath. 24: 1383–1384, 1934.

[1] Rosen, H. R. Morphological notes together with some ultrafiltration experiments on the crown-gall pathogene, *Bacterium tumefaciens.* Mycologia: 18: 193–205. 1926.

[2] Lieske, Rudolph. Untersuchungen über die Krebskrankheit bei Pflanzen, Tieren und Menschen. Centralbl. f. Bakt. etc. Abt. I. Originale 108: 118–146. 1928.

[3] Schätzel, Karl. Beiträge zur Morphologie und Physiologie des bakteriellen Pflanzenkrebserregers. Phytopath. Zeitschrf. 5: 251–273. 1933.

[4] Brown, Nellie A. A gall similar to crown gall, produced on Gypsophilla by a new bacterium. Jour. Agr. Res. 48: 1099–1112. 1934.

time potato-dextrose-peptone agar plates were streaked with a loopful of filtrate and subsequently incubated at 28° C. Agar plates developed no visible colonies in any case, while tubes with nutrient broth became turbid after 48 hours of incubation. Table 1 presents data on filtration of the milk cultures. It is apparent that the particles that constitute a filtrable form of the fire-blight organism can pass through Berkefeld V and N and Chamberland L_3 filters.

TABLE 1.—*Filtrability of Erwinia amylovora grown in skimmed milk*

Kind of filter	Incubation period (days):							
	7		12		24		36	
	Number of tubes:							
	+	−	+	−	+	−	+	−
Berkefeld V	10	0	10	0	10	0	12	0
Berkefeld N	10	0	10	0	10	0	10	2
Chamberland L_3	10	0	15	0	12	3	25	1

Smears of *Erwinia amylovora* made from the milk culture, when stained with thionin and methylene blue, revealed organisms with very dark-stained, round polar bodies. After 24 days, long chains were numerous in the cultures. All cultures obtained from the filtrates were found to be pathogenic on green pear fruits.

The same type of experiment was performed with the soft-rot organism, *Erwinia carotovora* (L. R. Jones) Holland. In the case of this organism the filtrability, again absent in bouillon, was demonstrated when the organism was grown in milk. As soon as the organism produced a considerable quantity of acid as a result of splitting milk sugar, no filtrability could be detected. Although the exact pH at which filtrability disappears was not determined, it was noted that cultures that had reached pH 6.6 no longer yielded filtrable forms.

Thus it appears that the filtrability of *Erwinia amylovora* and *E. carotovora* may be obtained under laboratory conditions by use of a special medium. Hydrogen-ion concentration of the medium may be a limiting factor in this phenomenon.

Attempts to get a filtrable stage of the 2 mentioned organisms from parts of the plants affected by them were unsuccessful.—P. A. ARK, Division of Plant Pathology, University of California, Berkeley, California.

Soggy Breakdown of Winter Banana Apples.[1]—Attention has been called to the similarity of the diseases known in the literature as soft scald and soggy breakdown of apples, as these occur on the varieties Wealthy and Golden Delicious.[2, 3, 4] In these two varieties the superficial appressed blemished areas typical of the soft-scald type of the disease may be found not only in the same storage lots of fruit, but on specimens that also have the typical internal deep-seated, soggy breakdown areas within the cortex region. That these two types of breakdown occur in the same storage lots under similar treatment and simultaneously on the same as well as in different specimens, suggests that they are identical.

Additional evidence indicating that this is the case has been obtained by recent storage experiments with the variety Winter Banana. In these experiments the apples were harvested on the usual commercial date and handled and stored in much the same manner as in investigations previously reported.[2, 5] Some lots of fruit were stored within 24 hours of picking, while others were delayed at 4-day intervals at a temperature of 50° F. before storing at 31° and 36° F.

All lots of fruit developed the low-temperature breakdown at 31° F., but those stored immediately and within the 5- and 9-day interval after picking were much more affected than those given longer delayed treatment. As with other varieties in other studies, no soggy breakdown occurred on this variety at 36° F. These results are similar to those obtained previously with Golden Delicious and Grimes Golden.[2, 5]

The appearance of the two types of breakdown occurring in this variety is shown in figures 1 and 2. In figure 1 is shown the broken down areas of the cortical portion of the apple, which are typical for the soggy breakdown type earlier described on Grimes Golden (*loc. cit.*). Figure 2 shows the superficial type of the disease, with the characteristic appressed and sometimes depressed blemishes, which resembles the soft-scald type common on Jonathan, Golden Delicious, Wealthy, and Northwestern Greening. Transverse and longitudinal sections of affected apples, however, (figure 2) frequently exhibit the same deep-seated broken-down areas as those illustrated in figure 1. Moreover, the superficial areas frequently coalesce with the deeper-seated soggy breakdown areas. The question then arises as to

[1] Journal Paper No. J-259 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 279.

[2] Plagge, H. H., and Maney, T. J. Soggy breakdown of apples and its control by storage temperature. Iowa Agr. Exp. Sta., Res. Bul. 115. 1928.

[3] Plagge, H. H. A study of soggy breakdown and some related functional diseases of the apple. Proc. Amer. Soc. Hort. Sci., 26: 315–318. 1929.

[4] Plagge, H. H., Maney, T. J., and Pickett, B. S. Functional diseases of the apple in storage. Iowa Agr. Exp. Sta., Bul. 329. 1935.

[5] Plagge, H. H. Effect of storage temperature on soggy breakdown of Golden Delicious apples. Proc. Amer. Soc. Hort. Sci., 25: 298–300. 1928.

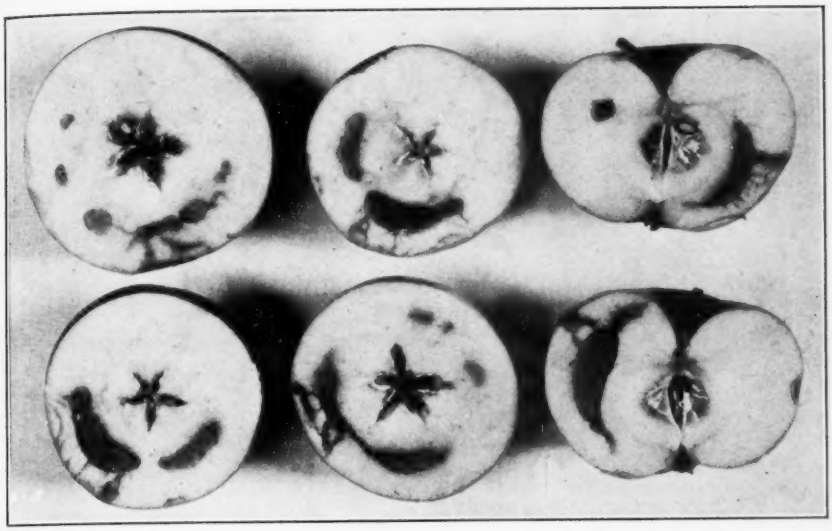

FIG. 1. Soggy breakdown in Winter Banana Apple.

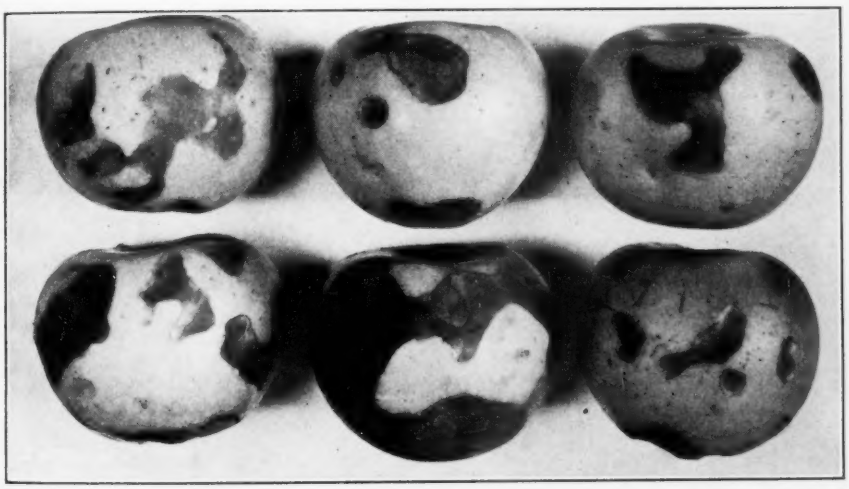

FIG. 2. Soft scald type of soggy breakdown of Winter Banana Apple.

the limitations of each type of disorder. The appearances of the diseased tissue of both types of the disorder, in all the varieties thus far studied, are similar in color, taste, and consistency. The results of this study with Winter Banana and other varieties (*loc. cit.*) have led the authors to conclude that the two types of low-temperature diseases herein illustrated and described are identical.—H. H. PLAGGE and T. J. MANEY, Department of Horticulture, Iowa State College, Ames, Iowa.